integrator/plotter: Shimadzu
Chromatopac GR, B,
Shimadzu GmbH,
D-4000 Düsseldorf

hydrogen,
nitrogen,
synthetic air,
Linde AG,
D-5000 Köln

Procedure

| | |
|---|---|
| Analysis: | approx. 1 ul injected directly into capillary column at ambient temperature |
| Gas chromatography | |
| Column: | 30 m x 0.32 mm inner diameter, fused silica deactivated with polysiloane |
| Stationary phase: | wall coated, DB-5 (a), film thickness: 0.25 nm |
| Carrier gas and column head pressure: | hydrogen, 0.8 bar (HDN) (corresponding linear velocity: 55 cm/sec at 55 degrees centigrade) |
| Make-up gas and flow rate: | nitrogen, 30 ml/min |
| Oven temperature: | temperature program: 0.5 min at 55 degrees centigrade, rate 10 degrees centigrade/min up to 260 degrees centigrade, 10 min at 260 degrees centigrade |
| Injector temperature: | ambient, upper part cooled with air |
| Detector temperature: | 325 degrees centigrade |
| Computation: | gas chromatographic determination of a standard solution of several fatty acid methyl esters, determination of relative response factors using the internal standard method |
| Scientific version: | SOP BC 179/2 |
| Text version: | 17.Aug.83 |

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(a) non extractable stationary phase = cross linked and chemically banded silicone containing 5 0/0 phenyl and 95 0/0 methyl groups

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## 4.21 Preparation of Protein for SDS Polyacrylamide Electrophoresis

Principle: dissociation of protein subunits with a sulfhydryl compound (beta-mercaptoethanol) and denaturation as well as surface coating with a negatively charged detergent (SDS)

Time

Sampling: on day 22

Preparation: within 7 d after sampling

Sample material and quantity: FLC homogenate, approx. 1E6 macrophages

Results expressed in: -

Equipment:

micro vials: type "Eppendorf", polypropylene, no. 3810,
thermostat: type "Eppendorf", no. 3401,
Netheler und Hinz GmbH,
D-2000 Hamburg 65

whirlmix: no. 34526,
Cenco Deutschland GmbH,
D-5667 Haan

analytical balance: model 2001 MP,
Sartorius GmbH,
D-3400 Göttingen

pH meter: PW 9409,
Philips GmbH,
D-3500 Kassel

magnetic stirrer: Ika-Combimag RCO,
Janke und Kunkel GmbH und Co. KG,
D-7813 Staufen

Chemicals:

disodium hydrogen phosphate-2-hydrate, no. 6580,
sodium dihydrogen phosphate-1-hydrate, no. 6346,

beta-mercaptoethanol, no. 805740,
glycerol, no. 4094,
bromophenol blue, no. 8122,
E. Merck,
D-6100 Darmstadt 1

sodium dodecyl sulfate (SDS),
no. 20760,
Serva Feinbiochemica GmbH und Co. KG,
D-6900 Heidelberg 1

iodoacetamide, no. I 6125,
Sigma Chemie GmbH,
D-8028 Taufkirchen

SDS phosphate buffer:
9.75 mmol phosphate buffer/l with
25 ml beta-methaptoethanol/l and
25 g SDS/l

final pH: 7.0

Procedure:

protein incubated for 3 min at 100 degrees centigrade in SDS phosphate buffer, pH 7.0. Thereafter addition of bromphenol blue as tracking dye and glycerol to increase sample density. Further incubation with iodoacetamide at 37 degrees centigrade for 15 min to prevent aggregation of subunits

final concentration of components in incubation mixture:

| | |
|---|---|
| protein | 0.5 g/l |
| SDS | 12.5 g/l |
| phosphate buffer, pH 7.0 | 4.88 mmol/l |
| beta-mercaptoethanol | 12.5 ml/l |
| bromophenol blue | 50 mg/l |
| glycerol | 250 ml/l |
| iodoacetamide | 60.4 mmol/l |

storage at minus 20 degrees centigrade, stability unlimited

Scientific version: SOP BC 131/3
Text version: 23.Aug.84

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## 4.22 SDS Polyacrylamide Gel Electrophoresis

Principle: separation of negatively charged complexes of proteins with a detergent (SDS) acccording to their relative molecular mass in an electrical field across a vertical polyacrylamide gel (separation gel: 125 g/l (= 12.5 0/0) acrylamide, stacking gel: 40 g/l (4 0/0) with defined pore size

Time

Sampling: on day 22

Determination: within 6 months after sampling

Sample material and quantity: protein SDS complexes, 5 to 40 ul equiv. to 2.5 to 20 ug protein/slot

Results expressed in: -

Equipment:

magnetic stirrer: Ika-Combimag RCO,
Janke und Kunkel GmbH und Co. KG,
D-7813 Staufen

pH meter: PW 9409,
Philips GmbH,
D-3500 Kassel

glass cell: Desaga Doppeltrennzelle,
thickness: 1.5 mm, width: 220 mm,
length: 110 mm, gel volume: 37 ml
electrophoresis apparatus: system
Havana,
Desaga GmbH,
D-6900 Heidelberg 1

power supply: no. 2103,
LKB Instruments,
D-8032 Gräfelfing

thermostat: Thermostar RM3,
Messgerätewerk Lauda, Dr. R. Wobser KG,
D-6970 Lauda-Königshofen

Chemicals:

acrylamide, no. A 8887,
N,N'-methylene-bis-acrylamide (Bis), no. M 7256,
trizma base (Tris), no. T 1503,
N,N,N',N'tetramethylethylendiamine (TEMED), no. T 8133,
glycine, no. G 7126,
Sigma Chemie GmbH,
D-8028 Taufkirchen

ammonium persulfate, no. 13375,
sodium dodecyl sulfate (SDS), no. 20760,
Serva Feinbiochemica GmbH und Co. KG,
D-6900 Heidelberg 1

sucrose, no. 7651,
EDTA, no. 8418,
E. Merck,
D-6100 Darmstadt 1

stacking gel composition (40 g/l):

| | | |
|---|---|---|
| acrylamide | 0.561 | mol/l |
| Bis | 6.89 | mmol/l |
| sucrose | 0.873 | mol/l |
| ammonium persulfate | 1.16 | mmol/l |
| TEMED | 1.33 | ml/l |
| Tris buffer, pH 6.8 | 0.125 | mol/l |
| SDS | 3.45 | mmol/l |
| EDTA | 2.01 | mmol/l |

separation gel composition (125 g/l):

| | | |
|---|---|---|
| acrylamide | 1.76 | mol/l |
| Bis | 21.6 | mmol/l |
| ammonium persulfate | 0.583 | mmol/l |
| TEMED | 0.665 | ml/l |
| Tris buffer, pH 8.8 | 0.374 | mol/l |
| SDS | 3.46 | mmol/l |
| EDTA | 2.04 | mmol/l |

electrode buffer composition:

| | | |
|---|---|---|
| Tris | 50 | mmol/l |
| glycine | 0.383 | mol/l |
| SDS | 3.47 | mmol/l |
| EDTA | 2 | mmol/l |

final pH (a): 8.8



(a) in absence of SDS

| | |
|---|---|
| Procedure: | glass cells placed in electrode buffer at 9 degrees centigrade<br>current for 2 glass cells: 48 mA for 1 h, afterwards 96 mA<br>tracking dye velocity: approx. 2.5 cm/h |
| Scientific version: | SOP BC 142/5 |
| Text version: | 11.Apr.84 |

4.23 Silver Staining of Proteins (Bio-Rad Method)

| | |
|---|---|
| Principle: | formation of complex between silver salt and fixed proteins, development of gray to brown color by developer containing paraformaldehyde |
| Time: | indefinite after fixation of protein in gel |
| Sample material and quantity: | protein SDS complexes, 5 to 40 ul equiv. to 0.2 to 1 ug protein/slot |
| Equipment: | magnetic stirrer: Ika-Combimag RCO, Janke und Kunkel GmbH und Co. KG, D-7813 Staufen<br>diffusion destainer, no. 146340, Desaga GmbH, D-6900 Heidelberg 1 |
| Chemicals: | propanol-2, no. 9634, acetic acid, no. 62E, ethanol, no. 983, E. Merck, D-6100 Darmstadt 1 |

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silver stain kit, no. 161-0443,
Bio-Rad Laboratories GmbH,
D-8000 München 50

Procedure:

1st fixation: 1 time for at least 60 min in 250 ml propanol-2/l, 100 ml acetic acid/l

2nd fixation: 2 times for at least 30 min in 100 ml ethanol/l, 50 ml acetic acid/l

oxidation: 1 time for 30 min in 100 ml oxidizer concentrate/l

washing: 3 times for at least 60 min in bidistilled water

staining: 1 time for 30 min in 100 ml silver reagent/l

washing: 1 time for at least 10 min in bidistilled water

1st developing: 1 min in developer solution under constant stirring

2nd developing: 2 times approx. 5 min in developer solution until maximal stain develops

stopping: immediate addition of 50 ml acetic acid/l to last developer solution until no more gas is formed, approx. 5 min

above procedure as suggested by Bio-Rad in accordance with Merril, C.R., Goldman, D., Sedman, S.A., Ebert, M.H., Science 211 : 1437-1438 (1981)

Scientific version: SOP BC 234/1
Text version: 23.Aug.84

## 4.24 Staining of Proteins (Coomassie Brilliant Blue Method)

| | |
|---|---|
| Principle: | binding of dye to fixed proteins, removal of excess dye by diffusion |
| Time: | fixation immediately after electrophoresis to prevent diffusion of protein subsequently stained |
| Sample material and quantity: | proteins in polyacrylamide gels |
| Results expressed in: | - |
| Equipment: | diffusion destainer, no. 146340, Desaga GmbH, D-6900 Heidelberg 1 |
| Chemicals: | propanol-2, no. 9634, acetic acid, no. 62E, Coomassie Brilliant Blue R250, no. 12553, E. Merck, D-6100 Darmstadt 1<br><br>methanol, no. 8045, Baker Chemikalien, D-6080 Gross-Gerau |
| Procedure: | fixation: for at least 15 min in 250 ml propanol-2/l, 100 ml acetic acid/l<br><br>staining: 30 min in 5 g Coomassie Brilliant Blue/l, 500 ml methanol/l and 100 ml acetic acid/l<br><br>destaining: 2 to 3 days in 100 ml propanol-2/l, 100 ml acetic acid/l with multiple exchange of destaining solution |
| Scientific version: | SOP BC 24/3 |
| Text version: | 21.Feb.84 |

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## 4.25 Evaluation of Stained Proteins

| | |
|---|---|
| Principle: | photometric determination of intensity of protein stain along the electrophoretic separation distance, integration of peak area |
| Time: | within 2 weeks after staining of polyacrylamide gels |
| Sample material and quantity: | Coomassie Brilliant Blue R250-stained polyacrylamide gels |
| Results expressed in: | peak area (arbitrary units) |
| Equipment: | dual wavelength scanner: model CS-910, Shimadzu Europe, D-4000 Düsseldorf<br>integrator: LDC 301 with printer/plotter, Milton Roy Deutschland GmbH, D-6467 Hasselroth 2 |
| Chemicals: | - |
| Procedure | |
| Photometric scanning: | stained gels positioned in the beam of dual wavelength scanner beam focused in the middle of gel slot<br>sample wavelength: 560 nm<br>reference wavelength: 400 nm<br>mode: absorbance,<br>slit size: 1.7 mm x 0.15 mm,<br>scan speed: 20 mm/min |
| Integration: | fix mode |
| Scientific version: | SOP BC 54/2 |
| Text version: | 21.Feb.84 |

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## 5 STORAGE OF MATERIALS AND RECORDS

Slides and computer print-outs are stored in our archives for at least 5 years. They can be claimed by the client.

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# 6 RESULTS AND DISCUSSION

## 6.1 Text

### 6.1.1 Biochemical parameters

The long range goal of studies such as this one is to find biochemical markers for specific FLC types, e. g. macrophages, granulocytes and lymphocytes, and/or subpopulations of a single FLC type, e. g. bacteriologically active macrophages. As classical biochemical methods generally require homogenization of tissue, the previous identification and separation of specific cell population is a prerequisite to the assignment of any marker to a given cell type.

Flow cytometric methods may one day replace classical biochemical methods by analyzing single cells in suspension individually or at least by sorting out specific subpopulations for further biochemical analysis. With these thoughts in mind, 3 classical biochemical methods were also included as parameters in this study. These methods are still under development and are especially limited by their determination in homogenates of the crude pools containing various cell types described in the following chapters on microscopic and flow cytometric methods. The short-term goal here is to find their detection limits and test for their general applicability in FLC studies.

#### 6.1.1.1 FLC protein pattern in SDS-PAGE

The 1st problem in developing a small scaled electrophoresis method lies in the determination of small amounts of protein (.LT.1 microgram) in solutions containing substances which can cause artifacts or high blanks, e. g. SDS. This problem has now

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Large Differences in the morphology of FLC in the various pools and groups could be seen microscopically from cytocentrifuge preparations (see BC Figures 1 to 9). These effects appeared to be dependent on the lavage method and/or the type of smoke exposure. In the following various methods have been tested for their usefulness as an objective and quantitative measure of these changes.

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been resolved by the adaption of the method of where protein is precipitated (Schaffner and Weissman, 1973), washed and determined photometrically by dye binding (see ... METHOD). With this method protein can be determined from almost any solution in nanogram amounts.

The protein determinations indicate that 1E6 macrophages/milliliter yield approx. 0.1 milligram protein/milliliter (see BC TABLES ... and ...). Except for the increase due to granulocytes in 1-GR, no pool dependent variations were found. A comparison 1-GR and 2-GR to control, however, revealed an approx. 2-fold increase in protein for both groups relative to 0-GR (see BC TABLE ..). At least part of the increase in the 1-GR can be explained by the presence of the large number of granulocytes, however, the increase for 2-GR appears to be due neither to granulocyte or macrophage number nor an increase in macrophage size (see microscopic and flow cytometric parameters ...).

Due to the low concentration of FLC protein in samples after washing only a maximum of approx. 5 micrograms protein per slot could be applied to the gel. This amount of protein was too small to allow quantification of the smaller components of the protein pattern when stained with Coomassie Blue (see BC TABLE .. and BC FIGURE ..). Using this method a decrease in a protein of approx. 45000 daltons was found for MS and SS-exposed groups relative to control, while another protein of approx. 15000 daltons demonstrated the inverse effect (see BC TABLE .. and BC FIGURE ..).

As the concentration of small samples is tedious and accompanied by large losses, the recently described silver staining method of Merril et al. (1981) was tested on these samples. This protein stain is 10 to 50-fold more sensitive than Coomassie Blue (see BC FIGURE ..) and offered the possibility of quantitating some of the smaller FLC protein components. With this method the decrease in a protein at or about 45000 daltons was confirmed but the increase at or about 15000 daltons was not reproduced.

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The largest change seen after silver staining of FLC proteins was an increase in a protein of approx. 12000 daltons in treatment groups. This increase was 4-fold for 1-GR and 7-fold for 2-GR, and appears to correlate with a change in FLC protein pattern seen in a previous study (a). A small decrease in the amount of an approx. 16000-daltons component was also found for 1-GR and 2-GR relative to 0-GR, however changes in this molecular weight range could also be due to hemoglobin from contaminating erythrocytes (see flow cytometer differential counting).

From the above it appears that there are major changes in the protein pattern of FLC after smoke exposure, and silver staining increases the sensitivity of this method so that fractions of FLC subpopulations, i. e. 1E4 FLC which could be obtained by sorting in a flow cytometer, might be studied.

6.1.1.2 FLC phospholipids and fatty acids

Due to high blanks and low sensitivity of phospholipid determination based on an anorganic phosphorus assay, the amount of phospholipid in FLC could only be determined for 5 pools (see BC TABLE 9). This parameter also reflects the problem of reference point in crude FLC fractions. The number of macrophages as a reference do not allow for the contribution of granulocytes, while the smaller size of granulocytes is neglected when the number of FLC is taken as a reference. Thus protein appears to be the best reference point and should be determined routinely for all fractions in future studies. Despite the small number of data and large variations, an extrem elevation of phospholipid in FLC, as reported for chlorphentermine by phospholipidosis (Reasor, 1983) after smoke exposure would not appear probable. This ~~suggested finding~~

(a) see REFERENCES: INBIFO study A 0500/3056 (FLC substudy)

is also in agreement with another smoke exposure study reported (De Lucia, 1982). With a detection limit of 5E6 macrophages, the determination of phospholipid using the methods described does not appear promising for future studies.

As a capillary gas chromatographic method for the analysis of fatty acid methyl esters (FAME) was available ~~in the analytical chemistry department~~ and this method is extremely sensitive (a), an effort was made to determine the fatty acid content and pattern in the remaining samples.

As a measure of phospholipid, the total integrated area of FAME per 1E6 FLC in each sample was determined. The mean of all weighted pools for this parameter in the 0-GR was 4.2 ± 0.6 (N = 6) units FAME total area per 1E6 FLC (see BC TABLES 10 and 11). The mean value was decreased in the 1-GR to 0.6-fold relative to the 0-GR, while the mean value for the 2-GR was similar to control, 1.1-fold. This decrease in the total amount of FAME found per FLC in the 1-GR, however, is probably mostly due to the smaller contribution of granulocytes per cell due to their smaller size.

In addition to total FAME the amounts of palmitic acid methyl ester (PAME) and stearic acid methyl ester (SAME) were determined. The mean value for PAME of weighted pools for 0-GR was 29.4 (N = 6) micrograms per 1E6 FLC (see BC TABLES 12 and 13). The same parameter for the 1-GR yielded a value of 23.0 or 0.8-fold of control (N = 3). The 1-GR also demonstrated an increasing trend with increasing number of cycles. This effect could be explained by the decrease in proportion of granulocytes ~~while a similar trend seen for pools 5, 6 and 7 for the single determinations of 2-GR could not be explained by the same~~. The small number of data especially for 2-GR, however, do not allow a definitive interpretation of these effects.

---

(a) detection limit for total fatty acid methyl ester (FAME) pattern from .LT.1E3 macrophages

The mean value for SAME of weighted pools for the 0-GR was 10.7 ± 0.4 (N = 5) micrograms per 1E6 FLC (see BC TABLES 14 and 15), or approx. 0.3-fold of PAME for control FLC. This parameter yielded values of 7.0 ± 0.4. (N = 4) and 10.9 ± -. (N = 2) for the 1-GR and 2-GR respectively. ~~Thus there was no large difference seen between PAME and SAME for the various groups for the mean values.~~

For the ratio of PAME (16 : 0) to SAME (18 : 0) in the individual pools ~~however,~~ there was a difference between early and late cycles (see BC TABLE 16). The mean ratio (16 : 0/18 : 0) for all groups for pools 1, 3 and 5 was 1.6-fold of that found for pools 2, 4, 6 and 7 (see BC TABLE 17 and BC Figure ). As the ratio (16 : 0/18 : 0) content of surfactant is very high in comparison to cellular membrane lipids (Spalding, 1983), this difference in FAME probably is related to the presence of surfactant in FLC either as non-specifically bound material on the cell surface, or as phagocytized material (Eckert, 1983). Thus despite the incomplete data set due to lack of material, FAME analyses of FLC appear to be of interest in future studies.

Disc.

### 6.1.1.3 Acid phosphatase activity (ACP)

ACP activity of rat pulmonary macrophages has been reported to be increased by cigarette smoke exposure (Martin, 1973). A fluorometric method has been developed at INBIFO for this parameter (a), but no comparison of control and smoke-exposed FLC was made in this previous study; ~~Thus~~ therefore, this parameter was included in the present study.

Although this method is very sensitive, with a detection limit of approx. 2E4 FLC, the day to day variations were as much as 4-fold for the control (see BC TABLE 18). The mean ACP for all pools in the 3 experiments for 0-GR was 257 ± 41 units per 1E6

(a) see REFERENCES: INBIFO study A 0500/3052

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FLC. The same values for the 1-GR and 2-GR were 313 ± 42 and 547 ± 61 respectively (see BC TABLES 18 to 24). The mean ratio of treatment groups versus control group for each pool and experiment were also evaluated in an effort to compensate for the day to day variations (see BC TABLE 22). The mean ratio for 1-GR for all pools in the 3 experiments was 1.9 ± 0.8 relative to control, indicating at least some induction of ACP after exposure to MSS. The mean ratio for 2-GR for all pools in experiment 3 was 1.3 ± —. In consideration of the 4-fold lower dose for SSS, these data could indicate that no extreme difference between SSS and MSS should be expected in the induction of ACP. The confirmation of such an interpretation, however, will require more data at various doses.

6.1.2 FLC distribution and number

As hemocytometer counts from lavage medium have been shown in a previous study (a) to be inaccurate due to the low concentration of FLC, only cytocentrifuge preparations from lavage medium were made and evaluated for comparison to preparations from resuspension medium in this study.

Although the lack of absolute macrophage number did not allow the calculation of weighted means for the various lavage media, some information can be gained by the comparison of individual pools.

In cytocentrifuge preparation from lavage medium and resuspension medium, 0-GR FLC consisted of approx. 98 percent macrophages, 1 percent granulocytes and 1 percent lymphocytes (see BC TABLES 23 to 34 and BC FIGURES ..). A slight increase in the number of granulocytes in FLC of 0-GR was seen when PBS plus BSA with or

? without or with

ist dies unsere übliche Schreibweise

(a) see REFERENCES: INBIFO study P 0500/3097

[Hier heißt sich "....." u. "INBIFO" Engl. ... ist gleich]

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without PBS plus calcium and magnesium were used as lavage medium instead of PBS alone. This effect of lavage medium on the relative number of granulocytes in FLC was confirmed by the data for 2-GR. In this group, the relative number of granulocytes increased from approx. 13 percent in pool 1 to 31 percent in pool 3 and 22 percent in pool 5 for lavage medium (see BC TABLE 28 and BC FIGURE ..).

The respective values for relative number of granulocytes for resuspension medium were 8 percent, 20 percent and 27 percent (see BC TABLES 30 and BC FIGURE ..). A similar decrease in the relative number of granulocytes for 1-GR in pool 1 from 72 percent in lavage medium to 60 percent in resuspension medium indicate a specific loss of granulocytes during harvest when PBS alone was the lavage medium. Due to their lack of adherence, granulocytes were concentrated in the 1st 3 cycles, however, this effect was not increased as much as expected by the addition of calcium and magnesium to PBS (see BC TABLES 28 and 30 and BC FIGURES ..).

The relative number of lymphocytes was independent of group, pool and medium (see BC TABLES 31 to 34 and BC FIGURES ..).

The means of weighted pools for absolute number of macrophages in the 0-GR were 1.4 ± 0.3E6, 2.1 ± 0.3E6 and 3.3 ± 0.1E6 per rat for PBS, PBS plus BSA and PBS plus BSA prelavaged with PBS plus calcium and magnesium respectively. The respective values for the 1-GR were 1.0 ± 0.1E6, 2.1 ± 0.1E6 and 2.8 ± 0.5E6 (see BC TABLES 35 and BC FIGURE ..). Thus both sham and MSS-exposed rats demonstrated significant differences in the number of macrophages lavaged depending on the lavage medium used but independent of their treatment (see BC TABLES 35 and 36 and BC FIGURES ..). The pools 1 and 5 also demonstrated a significant decrease in the absolute number of lavaged macrophages in the 1-GR relative to 0-GR which could be interpreted as an increase in macrophage

adherence due to MSS exposure. Similar effects on absolute macrophage number for 0-GR and 1-GR were also seen in a previous study (a). The decrease in absolute macrophage number also seen in the same study (a) for 2-GR, was reproduced in this study for PBS alone and PBS plus BSA prelavaged with PBS plus calcium and magnesium. The weighted means were 0.4E6 and 2.3E6 respectively. For PBS plus BSA without prelavage, this parameter for the 2-GR was elevated relative to 0-GR and 1-GR, i. e. weighted mean of 2.6E6 macrophages/rat.

For this as well as all other parameters, however, one must consider that these are single determinations in this study for SSS exposure, and can only be used as points of interest in future studies.

Another interesting observation on absolute macrophage number was that despite the unexpectedly large number of macrophages removed by prelavage with PBS plus calcium and magnesium, the number of macrophages recovered in the following 10 cycles with PBS plus BSA was increased for 0-GR and 1-GR relative to the same number of cycles with PBS plus BSA without prelavage (see BC FIGURE ..).

The determination of absolute granulocyte number in a hemocytometer with unstained viable cells has been shown in a previous study (a) to be extremly inaccurate. Thus this determination was not performed in this study. Changes in the relative number of cell populations, however, are interdependent and can be misleading or at best difficult to interpret. Therefore, the absolute number of granulocytes was calculated from relative values for macrophages and granulocytes in cytocentrifuge preparations and the absolute number of macrophages in resuspension medium (see BC TABLES 37 and 38 and BC FIGURE ..). Both the larger relative

(a) see REFERENCES: INBIFO study P 0500/3097

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number of granulocytes in early cycles and the decrease in relative number of granulocytes recovered with PBS were confirmed by calculated absolute numbers. Further, more granulocytes were recovered by 3 cycles of PBS plus calcium and magnesium as in 10 cycles with PBS alone.

(BC TABLE 38)

6.1.3 Viability of macrophages

The viability of FLC determined immediately after harvest by dye exclusion appeared to be good, 94 percent or more viable macrophages (see BC TABLES 39 and 40 and BC FIGURES ..). Closer observation, however, revealed that pool 1 of experiment 1 for 0-GR and 1-GR were the lowest values found (see BC TABLE 39). The means of pools (see BC TABLE 40) also revealed a decrease in viability for

(1) early versus late cycles,

(2) smoke-exposed versus sham control groups

and

(3) PBS versus other lavage media.

These differences, however, were too small to be convincing despite the small variation seen (see BC FIGURE ..). The calculation of the absolute number of nonviable macrophages per rat, however, ~~lended support to the significance of~~ supported these observations (see BC TABLE 41 and BC FIGURE ..). The only exception to this statement was the smaller number of nonviable macrophages found for 2-GR lavaged with PBS. This discrepancy is probably related to the extremely low number of macrophages recovered in this group. The problems associated with this "acute" parameter have been discussed in detail in a previous report (a).

(a) see REFERENCES: INBIFO study P 0500/3097

6.1.4 Number of multinucleated macrophages (microscopic method)

The number of multinucleated macrophages has been reported to be increased by smoke exposure in a previous study (a), therefore this parameter was recorded simultaneously during differential counting. Although the apparent variation in this parameter was small, the microscopic data should be considered preliminary as only a maximum of 500 macrophages were examined and the relative number of multinucleated cells was very small.

This parameter demonstrated no pool or lavage medium dependent variations. The mean of weighted pools for relative number of multinucleated macrophages in all 3 lavage media for 0-GR was 1.1 percent ± 0.1 (N = 8). This value for the 1-GR, however, was increased to 5.31 percent ± 0.5 (N = 8), while the 2-GR was intermediate, 2.8 percent ± 0.3 (N = 3) (see BC TABLES 43 and 44 and BC FIGURES ..).

The mean absolute number of multinucleated macrophages also confirmed this ranking. The values were 25E3 ± 5E3 (N=3), 103E3 ± 22E3 (N=8) and 48E3 ± 20E3 (N=8) for the 0-GR, 1-GR and 2-GR respectively (see BC TABLES 45 and 46, BC FIGURES ....). The variation, however, was larger for the absolute values. This is largely due to the large difference in macrophage number between the various lavage media.

6.1.5 Morphometry of macrophages

As changes in macrophage size and vacuolization have been reported to increase after smoke exposure (~~Sewiz~~ Lewis, et al. 1979, ....), the area of macrophages, nuclei and vacuoles was determined planimetrically from

(a) see REFERENCES: INBIFO study A 0500/3016

(b) ~~weighted means~~ of pools

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microphotographic slides of stained cytocentrifuge ~~sediments~~ preparations in this study.

The changes in macrophage morphology are easily observed by microscopic examination of cytocentrifuge preparations (see BC FIGURES 1 to 8...). The quantification of these parameters, however, is difficult, and very much dependent on the quality of the cell preparation. In agreement with viability data (see also flow cytometer method) cell preparations from lavage with PBS alone were of such poor quality that no morphometry could be made (see BC FIGURES 1 and 2).

The mean macrophage area of weighted pools from lavage with PBS plus BSA without and with prelavage were 210 square micrometers (N = 240) and 224 square micrometers (N = 469) respectively. The respective values for 1-GR were 365 square micrometers (N = 397), and for 2-GR, 259 square micrometers (N = 190) and 275 square micrometers (N = 230) (see BC TABLE 47). The difference for macrophage size between lavage media for 1-GR and the increase with cycle number for 2-GR correlated with an increase in nuclear area (see BC TABLES 47 and 49) and is considered to be due to unspecific swelling during handeling and/or cytocentrifuge procedure. The increase found in smoke-exposed groups was too large to be explained by these effects and has also been confirmed by flow cytometry.

The main change in macrophage morphology after smoke exposure [redacted] was the increase in vacuolization. The mean vacuole area of weighted pools from lavage with PBS plus BSA without and with prelavage were 0.6 square micrometers (N = 493) and 0.8 square micrometers (N = 1433) respectively (see BC TABLE 51 and BC FIGURE ..). The respective values for 1-GR were 2.2 square micrometers (N = 1038) and 2.3 square micrometers (N = 2840). This is equivalent to a 3 to 4-fold increase in mean vacuole area relative to the control. The increase in mean vacuole area for the 2-GR was

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much smaller than for 1-GR with a maximum of 1.7-fold relative to the control (see BC TABLE 51), however, the vacuole area per macrophage was increased considerably, 2 to 3-fold relative to the control (see BC TABLE 52, BC FIGURE .....). Thus SSS exposure increased the number but not so much the size of vacuoles. The increase in percent of macrophage area covered by vacuoles in the 1-GR was even larger, 5 to 6-fold of control, than the increase in size of vacuoles (see BC TABLE 53). Thus MSS exposure increased the number and the size of vacuoles.

## 6.1.6 Flow cytometry parameters

### 6.1.6.1 Evaluation Schemes

#### 6.1.6.1.1 Viability and esterase assay

FLC from smoke-exposed rats mainly consist of macrophages and granulocytes, which were found to be resolved to a limited degree by FWD (signal area) and AXL (signal height) see BC FIGURE ...). Enhanced resolution was, in some instances, achieved when the 2 parameters were correlated in a cytogram of FWD versus AXL (see (2), (3), and (6), BC FIGURE .. and BC FIGURE ..).

The 4 parameters involved in the viability and esterase assay, FWD, AXL, red and green fluorescence, may be evaluated in different ways. The simplest method of 1st differentiating between cell types using FWD and AXL (see (1), (2) and (5), BC FIGURE .. and BC FIGURE ..) followed by separation of viable and nonviable cells in the FWD versus AXL region (see (3), (4) and (6), BC FIGURE ..) was found to be in appropriate as nonviable cells were not exclusively in the same region as viable cells. Therefore, all signals were used to create a green versus red fluorescence cytogram (see (6), BC FIGURE .. and BC FIGURE ..). An FWD versus AXL cytogram (7) was then created only from cells in a specified region of the green versus red fluorescence (6). This cytogram

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provide a basis for future method optimization. Taking into consideration that the FLC samples analyzed by FCM and microscopy were processed in completely different ways, the consistency of the results was surprisingly good (see BC TABLE .. and BC FIGURES .. and ..). (very)

In many samples a sharp peak was seen in AXL histograms which corresponded to a cluster in region ... of the FWD versus AXL cytogram (see BC FIGURE ..). As no nucleated cells were found in this region, it was assumed that this peak may represent red blood cells. This interpretation is ~~strongly~~ supported by RBC counts from cytocentrifuge preparations (see BC TABLE ..).

### 7.1.7 Conclusions

Of the biochemical methods tested in this study, SDS-PAGE and acid phosphatase activity appear to be sensitive enough to be use for determinations in separate subpopulations of FLC. The acid phosphatase method, however, is based on detection of fluorescence product and can be easily adapted to FCM. ~~when~~ The single cell analysis of this parameter should yield even more information.

The determination of phospholipids in FLC have been shown to be too insensitive to be used extensively in FLC studies. The alternative method of FMAE pattern using capillary GC, however, appears sensitive enough to be promising for future studies on FLC lipids in small subpopulations.

The cellular composition of FLC was strongly influenced by lavage procedure. Generally, the relative number of granulocytes was larger in the 1st pool of each lavage. Supplimentation of the lavage medium PBS with BSA or calcium and magnesium also increased the proportion of granulocytes.

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The large increase in relative number of granulocytes after MSØ exposure and the small increase for SSØ exposure were in agreement with a previous study (a).

The absolute number of macrophages recovered was also increased by supplimentation with BSA. Although an unexpectedly large number of macrophages were recovered in the prelavage with PBS plus calcium and magnesium, the number of macrophages obtained in the following 10 cycles with PBS plus BSA was comparable to the same number of cycles without prelavage.

The number of macrophages recovered from rats exposed to MSØ was similar to that for controls as ~~already~~ seen in a previous study (a). The decrease in macrophage number after SSØ exposure was also reproduced for PBS alone and PBS plus BSA with prelavage. In the case of PBS plus BSA without prelavage, however, the number of macrophages recovered from the SS group was increased relative to control. As this was only a single determination, no interpretation is possible.

The absolute number of granulocytes confirmed the changes seen for relative number of the same, despite the large variations in macrophage number between lavage procedures and treatment groups.

Viability, determined microscopically, appeared to be lower in PBS alone than for other media. Especially the variation in viability was larger for this medium. The 1st pool of each lavage procedure also demonstrated a small decrease in viability relative to later pools.

For the microscopical determination of macrophage morphology, PBS lavaged samples were not used as the cell preparations were not well preserved. No other changes in macrophage morphology were seen between the

(a) see REFERENCES: INBIFO study P 0500/3097

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3 lavage procedures. There was, however, a definitive increase in macrophage size after smoke exposure. The larger effect of MS$ on macrophage size relative to SS$ for PBS plus BSA without prelavage was not confirmed in PBS plus BSA with prelavage.

The determination of FLC viability by FCM was shown to be practicable in this study. In addition to the information also obtained by the trypan blue method, this method can quantitate intermediate states indicating acute damage to FLC. As this method is also independent of subjective factors, it can and should replace the microscopic method in future studies. The effect of lavage media and smoke exposure were similar to the microscopic data.

The determination of nonspecific esterase activity of macrophages in the same assay, was essentially unimodal and demonstrated no difference between lavage procedure or treatment groups. A bimodal distribution was found in granulocytes for this parameter. Due to the poor resolution of the 2 subpopulations, however, an exact evaluation of these data is not yet possible.

Phagocytosis as determined by FCM was found to be a sensitive parameter for smoke exposure effects. An increase in phagocytosis relative to control was seen for MS$ and SS$. The small number of data available in this study, especially for SS$, were in agreement with a previous ~~larger~~ study (a). The analysis of granulocyte phagocytosis is a new and interesting aspect of this parameter. Due to the small number of granulocytes in the pools for phagocytosis from controls, no exact evaluation of this parameter was possible. The use of granulocyte enriched pools, e. g. pool 5, for such studies should be incorporated into future studies.

The values for DNA content of macrophages were shifted to higher values compared to granulocytes, possibly due to autofluorescence.

(a) see REFERENCES: INBIFO study P 0500/3097

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The determination of DNA distribution offers an opportunity to determine stages of proliferation and multinucleation of macrophages. Both proliferation and multinucleation of macrophages were significantly increased in MS$ exposed FLC. Variations and limited amount of data do not allow an interpretation of this parameter for SS exposed group.

Differential counts determined with FCM on the basis of AXL and FWD are in general agreement with microscopic data, and can be refined by the use of additional parameters such as DNA. Their inclusion in a given assay allows the separate analysis of macrophages and granulocytes in heterogenous FLC samples, and can replace the microscopic method in future studies.

The light scatter parameters AXL, RAS and FWD were used to analyze samples for subpopulations of macrophages for differing morphology. AXL demonstrated a significant increase after MS$ exposure relative to control. A smaller increase was seen for SS$ exposure. This effect probably correlates with an increase in macrophage size seen microscopically.

For RAS a large increase was also seen for MS$ exposure group relative to control. The SS$ exposed group was also significantly increased relative to control. This group, however, was also significantly lower in RAS than the MS group. Due to the lack of a suitable logarithmic amplifier, evaluation of this parameter was limited, however, it appears to be of potential interest as an assay for vacuolization.

FWD from the viability assay demonstrated no difference between the MS$ exposed group and control. For the SS$ exposed group, however, a small but significant increase in this parameter was seen for viable macrophages. The FWD histogram for this group

demonstrated a definite bimodal distribution, indicating a high FWD subpopulation of macrophages. The appearance of such a new subpopulation might be seen in correlation with the decrease in macrophage number for SS$ exposed rats. This effect could then be explained by the compensation of macrophage loss by recruitment of monocytes.

From the results of this study, it is apparent that handling is very critical for FLC parameters. By

(1) the replacement of microscopic determination of viability and differential counts by the FCM methods and
(2) the replacement of microscopic cell counting by a coulter counter at the site of lavage

the time from lavage to analysis or fixation should be reduced significantly.

For the application of FCM to FLC parameters the most important result of this study was the development of evaluation schemes including software for

(1) viability,
(2) phagocytosis and
(3) DNA assays.

Supplimentation of PBS as a lavage medium with BSA increased macrophage yield and viability. The number of lavaged macrophages was further increased by an additional prelavage with PBS plus calcium and magnesium. Although the viability for the latter was slightly decreased relative to PBS plus BSA without prelavage, this lavage procedure including prelavage is considered to be the best compromise for future studies. This procedure not only provides sufficient FLC for the investigation of various parameters, but also yields macrophage fractions with fewer contaminating granulocytes and RBC. In addition, the prelavage yields a fraction enriched in granulocytes which appear to be of special interest in the comparison of MS$ and SS$ as determined by FCM.

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SUBREPORT P 0500/3057 GD91 (R) A1 PS

## 6.2 Tables and Figures

| GROUP | EXPERIMENT NO. | PROTEIN (mg/l) POOL | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 0-GR | 1 | – | 53.1 | – | 93.1 | 89.6 | – | 45.3 |
| | 2 | – | 92.7 | – | 59.6 | – | 61.8 | 61.8 |
| | 3 | – | – | – | 114.8 | 123.5 | 78.3 | 88.7 |
| 1-GR | 1 | – | 79.2 | – | 72.2 | – | – | 92.7 |
| | 2 | – | 173.5 | – | 151.3 | – | – | 189.6 |
| | 3 | – | – | – | 187.8 | – | 138.7 | 125.3 |
| 2-GR | 3 | – | – | 100.9 | 181.8 | – | – | 114.8 |

BC TABLE 1

FLC-PROTEIN [illegible]

Remarks: amido black method

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SUBREPORT P 0500/3057 GD91 (R) A2 PS

| GROUP | STATISTICAL PARAMETER | PROTEIN ~~(mg/l)~~ POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | - | 2 | - | 3 | 2 | 2 | 3 |
| | M (mg/l) | | 72.9 | | 89.2 | 106.6 | 70.1 | 65.3 |
| | SE | | - | | 16.1 | - | - | 12.7 |
| | RSD (0/0) | | - | | 31.2 | - | - | 33.6 |
| 1-GR | N | - | 2 | - | 3 | - | 1 | 3 |
| | M (mg/l) | | 126.4 | | 137.1 | | 138.7 | 135.9 |
| | SE | | - | | 34.1 | | - | 28.5 |
| | RSD (0/0) | | - | | 43.1 | | - | 36.3 |
| 2-GR | x | - | - | 100.9 | 181.8 | - | - | 114.8 |

BC TABLE 2

FLC-PROTEIN, STATISTICAL PARAMETER

Remarks: amido black method

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SUBREPORT P 0500/3057 GD91 (R) A7 PS

| GROUP | EXPERIMENT | POOL | RELATIVE PEAK AREA (0/0) | | |
|---|---|---|---|---|---|
| | | | Mr (a) ← (DALTON 1E3) | | |
| | | | 45.5 | 16.0 | 15.0 |
| 0-GR | 1 | 2 | 32.5 | 27.6 | 0 |
| | | 4 | 19.5 | 26.8 | 27.3 |
| | | 7 | 21.1 | 39.0 | 0 |
| | 2 | 2 | 16.7 | 34.5 | 9.9 |
| | | 4 | 23.0 | 38.8 | 12.0 |
| | | 7 | 32.9 | 33.9 | 0 |
| | 3 | 4 | 10.9 | 55.9 | 0 |
| | | 7 | 29.5 | 48.9 | 0 |
| 1-GR | 1 | 2 | 17.2 | 36.6 | 11.7 |
| | | 4 | 14.6 | 32.2 | 15.9 |
| | | 7 | 23.2 | 39.7 | 8.6 |
| | 2 | 2 | 12.2 | 12.2 | 8.2 |
| | | 4 | 15.4 | 23.0 | 15.7 |
| | | 7 | 15.1 | 24.5 | 15.8 |
| | 3 | 4 | 10.1 | 33.7 | 13.7 |
| | | 7 | 13.6 | 49.7 | 0 |

BC TABLE ~~8~~ 3

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC PROTEINS, [illegible] COOMASSIE BLUE STAINING

Remarks: for Mr standards and amount of protein see BC FIGURE: values are from a single determination for each pool and day

(a) relative molecular mass

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SUBREPORT P 0500/3057 GD91 (R) A8 PS

MD

[an vorhergehende Tab. anhängen]

| GROUP | EXPERIMENT | POOL | RELATIVE PEAK AREA (0/0) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Mr (a) ⇐ (DALTON 1E3) | | | | |
| | | | ~~67.0~~ | 45.5 | ~~43.0~~ | 16.0 | 15.0 |
| 2-GR | 3 | 4 | ~~19.3~~ | 1.8 | ~~0~~ | 16.1 | 43.5 |
| | | 7 | ~~2.5~~ | 13.8 | ~~0~~ | 54.2 | 0 |

BC TABLE ~~B~~3 (continued)

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC PROTEINS, COOMASSIE BLUE STAINING

Remarks: for Mr standards and amount of protein see BC FIGURE: values are from a single determination for each pool and day

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(a) relative molecular mass

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SUBREPORT P 0500/3057 GD91 (R) A9 PS

| GROUP | STATISTICAL PARAMETER | RELATIVE PEAK AREA (~~0/0~~) Mr (a) (Dalton 1E3) 45.5 | 16.0 | 15.0 |
|---|---|---|---|---|
| 0-GR | N | 8 | 8 | 8 |
| | M (%) | 23.26 | 38.18 | 6.15 |
| | SE | 2.78 | 3.54 | 3.50 |
| | RSD (0/0) | 33.8 | 26.3 | 160.8 |
| 1-GR | N | 8 | 8 | 8 |
| | M (%) | 15.18 | 31.45 | 11.20 |
| | SE | 1.38 | 4.06 | 1.94 |
| | RSD (0/0) | 25.6 | 36.5 | 49.0 |
| 2-GR | N | 2 | 2 | 2 |
| | M (%) | 7.80 | 35.15 | 21.75 |
| | SE | - | - | - |
| | RSD (0/0) | - | - | - |

BC TABLE ~~B~~4

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC, STATISTICAL PARAMETERS, COOMASSIE BLUE STAINING

(a) relative molecular mass

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SUBREPORT P 0500/3057 GD91 (R) A3 PS

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| GROUP | EXPERIMENT | POOL | RELATIVE PEAK AREA (0/0) Mr (a) ← (DALTON 1E3) 44.2 | 20.0 to 19.0 | 18 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 2 | 17.4 | 9.7 | 8~~9~~.6 | 23.6 | 6.2 | 0 |
| | | 4 | 14.7 | 11.7 | 11.3 | 18.2 | 24.8 | 0.4 |
| | | 5 | 9.8 | 8.1 | 9.0 | 17.0 | 28.5 | 1.0 |
| | | 7 | 6.9 | 5.9 | 20.8 | 28.9 | 15.4 | 1.8 |
| | 2 | 2 | 10.3 | 12.5 | 12.7 | 23.0 | 15.7 | 1.8 |
| | | 4 | 14.3 | 11.6 | 11.4 | 21.9 | 16.5 | 0.8 |
| | | 6 | 17.7 | 8.5 | 9.2 | 23.5 | 15.2 | 1.5 |
| | | 7 | 18.4 | 10.8 | 10.6 | 24.3 | 9.0 | 0.4 |
| | 3 | 4 | 5.8 | 14.7 | 14.7 | 41.7 (b) | 3.2 (b) | 0.4 |
| | | 5 | 3.9 | 9.9 | 9.0 | 16.2 | 44.6 | 1.5 |
| | | 6 | 4.5 | 15.6 | 14.7 | 29.5 | 9.3 | 0.~~5~~3 |
| | | 7 | 6.4 | 17.3 | 15.2 | 39.6 (b) | 0 | .LT.0.2 (b) |

BC TABLE 35

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC PROTEINS, RESUSPENSION MEDIUM SILVER STAINING

Remarks: for Mr standards and amount of protein see BC FIGURE:
values are from a single determination for each pool and day

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(a) relative molecular mass
(b) not included in the calculation of means

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SUBREPORT P 0500/3057 GD91 (R) A4 PS

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| GROUP | EXPERIMENT | POOL | RELATIVE PEAK AREA (0/0) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mr (a) ← (DALTON 1E3) | | | | | |
| | | | 44.2 | 20.0 to 19.0 | 18.0 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
| 1-GR | 1 | 2 | 8.5 | 11.8 | 11.7 | 22.2 | 23.5 | 2.4 |
| | | 4 | 11.5 | 11.8 | 12.2 | 17.6 | 15.8 | 2.1 |
| | | 7 | 12.4 | 14.6 | 12.2 | 17.6 | 10.2 | .LT.0.1 (b) |
| | 2 | 2 | 11.1 | 12.2 | 11.5 | 15.4 | 19.3 | 5.9 |
| | | 4 | 10.3 | 12.9 | 13.2 | 19.1 | 19.1 | 5.7 |
| | | 7 | 7.8/7 | 8.5 | 12.2 | 18.8 | 16.0 | 4.8 |
| | 3 | 4 | 6.0 | 11.8 | 12.6 | 23.7 | 19.5 | 4.6 |
| | | 6 | 3.7 | 12.9 | 11.7 | 19.7 | 22.4 | 13.4 |
| | | 7 | 6.6 | 16.5 | 13.2 | 19.9 | 12.8 | 2.1 |

BC TABLE 35 (continued)

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC PROTEINS, SILVER STAINING

Remarks: for Mr standards and amount of protein see BC FIGURE:
values are from a single determination for each pool and day

(a) relative molecular mass
(b) not included in the calculation of means

SUBREPORT P 0500/3057 GD91 (R) A5 PS

mo.

| GROUP | EXPERIMENT | POOL | RELATIVE PEAK AREA (0/0) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mr (a) ← (DALTON 1E3) | | | | | |
| | | | 44.2 | 20.0 to 19.0 | 18 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
| 2-GR | 3 | 3 | 6.0 | 8.7 | 8.5 | 14.3 | 26.6 | 17.4 |
| | | 4 | 2.4 | 5.1 | 6.5 | 12.2 | 48.8 | 3.8 |
| | | 7 | 6.8 | 18.9 | 13.4 | 23.5 | 12.4 | 1.0 |

BC TABLE 25 (continued)

RELATIVE PEAK AREA FROM SDS-PAGE OF FLC PROTEINS, SILVER STAINING

Remarks: for Mr standards and amount of protein see BC FIGURE:
values are from a single determination for each pool and day

(a) relative molecular mass
~~(b) not included in the calculation of means~~

MO

SUBREPORT P 0500/3057 GD91 (R) A6 PS

| GROUP | STATISTICAL PARAMETER | RELATIVE PEAK AREA ~~(0/0)~~ Mr (a) ⟸ (DALTON 1E3) 44.2 | 20.0 to 19.0 | 18.0 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
|---|---|---|---|---|---|---|---|
| 0-GR | N | 12 | 12 | 12 | 10 | 11 | 11 |
| | M (%) | 10.84 | 11.36 | 12.~~35~~27 | 22.61 | 16.84 | 0.9~~2~~0 |
| | SE | 1.57 | 0.95 | 1.0~~7~~4 | 1.43 | 3.68 | ~~0.19~~ 0.20 |
| | RSD (0/0) | 50.1 | 29.0 | ~~28.4~~ 29.3 | 20.0 | 72.5 | ~~69.5~~ 72.7 |
| 1-GR | N | 9 | 9 | 9 | 9 | 9 | 8 |
| | M (%) | 8.6~~6~~4 | 12.56 | 12.28 | 19.33 | 17.62 | 5.13 |
| | SE | 0.97 | 0.73 | 0.21 | 0.83 | 1.44 | 1.31 |
| | RSD (0/0) | 33.~~5~~6 | 17.4 | 5.1 | 12.9 | 24.6 | 72.1 |
| 2-GR | N | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (%) | 5.07 | 10.90 | 9.47 | 16.67 | 29.27 | 7.40 |
| | SE | 1.35 | 4.13 | 2.05 | 3.47 | 10.59 | 5.06 |
| | RSD (0/0) | 46.3 | 65.7 | 37.5 | 36.1 | 62.7 | 118.5 |

BC TABLE ~~A~~ 6

~~MEAN~~ RELATIVE PEAK AREA FROM SDS-PAGE OF FLC, STATISTICAL PARAMETERS, SILVER STAINING

(a) relative molecular mass

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SUBREPORT P 0500/3057 GD91 (R) A10 PS

| GROUP | EXPERIMENT | POOL | ~~QUOTIENTS~~, RELATIVE PEAK AREA RATIO (a) Mr (b) ← (DALTON 1E3) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 44.2 | 20.0 to 19.0 | 18 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
| 1-GR | 1 | 2 | 0.49 | 1.22 | 1.~~22~~36 | 0.94 | 3.79 | - |
| | | 4 | 0.78 | 1.01 | 1.08 | 0.97 | 0.64 | 5.25 |
| | | 7 | 1.80 | 2.47 | 0.59 | 0.61 | 0.66 | .LT.0.06 (c) |
| | 2 | 2 | 1.08 | 0.98 | 0.91 | 0.67 | 1.23 | 3.28 |
| | | 4 | 0.72 | 1.11 | 1.16 | 0.87 | 1.16 | 7.13 |
| | | 7 | 0.42 | 0.79 | 1.15 | 0.77 | 1.78 | 12.00 |
| | 3 | ~~2~~ 4 | 1.03 | 0.80 | 0.86 | 0.57 | 6.09 | 11.50 |
| | | ~~4~~ 6 | 0.82 | 0.83 | 0.80 | 0.67 | 2.41 | ~~26.80~~ 44.67 |
| | | 7 | 1.03 | 0.95 | 0.87 | 0.50 | - | .GT.10.5 (c) |

BC TABLE 7

RATIO OF RELATIVE PEAK AREA FROM SDS-PAGE OF FLC, SILVER STAINING

(a) treatment group versus control group
(b) relative molecular mass
(c) values not used to calculate mean quotient (see BC TABLE ...)

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SUBREPORT P 0500/3057 GD91 (R) A11 PS

| GROUP | EXPERIMENT | POOL | ~~QUOTIENTS~~, RELATIVE PEAK AREA RATIO (a) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mr (b) ← (DALTON 1E3) | | | | | |
| | | | 44.2 | 20.0 to 19.0 | 18 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
| 2-GR | 3 | 3 | - | - | - | - | - | - |
| | | 4 | 0.41 | 0.35 | 0.44 | 0.29 | 15.25 | 9.50 |
| | | 7 | 1.06 | 1.09 | 0.88 | 0.59 | - | .GT. ~~5.07~~ 5.00 (c) |

BC TABLE 7 (continued)

RATIO OF RELATIVE PEAK AREA FROM SDS-PAGE OF FLC, SILVER STAINING

(a) treatment group versus control group
(b) relative molecular mass
(c) values not used to calculate mean quotient (see BC TABLE ...)

SUBREPORT P 0500/3057 GD91 (R) A12 PS

no

| GROUP | STATISTICAL PARAMETER | RELATIVE PEAK AREA ~~(0/0)~~ ~~MEAN~~ RATIO (a) Mr (b)←(DALTON 1E3) 44.2 | 20.0 to 19.0 | 18.0 to 17.5 | 16.3 | 15.5 to 14.5 | 12.4 |
|---|---|---|---|---|---|---|---|
| 1-GR | N | 9 | 9 | 9 | 9 | 8 | 6 |
| | M (%) | 0.908 | 1.129 | 0.9~~60~~76 | 0.730 | 2.220 | ~~10.993~~ 13.972 |
| | SE | 0.136 | 0.174 | 0.0~~69~~77 | 0.056 | 0.664 | ~~3.457~~ 6.297 |
| | RSD (0/0) | 44.9 | 46.3 | ~~21.5~~ 23.8 | 22.9 | 84.6 | ~~77.0~~ 110.4 |
| 2-GR | N | 2 | 2 | 2 | 2 | 1 | 1 |
| | M (%) | 0.735 | 0.720 | 0.660 | 0.440 | 15.25 | 9.50 |
| | SE | - | - | - | - | - | - |
| | RSD (0/0) | - | - | - | - | - | - |

BC TABLE 8

~~MEAN~~ RATIO OF RELATIVE PEAK AREA FROM SDS-PAGE OF FLC, STATISTICAL PARAMETERS

(a) treatment group versus control group
(b) relative molecular mass

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SUBREPORT P 0500/3057 GD91 (R) A13 PS

| GROUP | EXPERIMENT NO. | POOL | PHOSPHOLIPID (ug/1E6 ~~microphy.~~ MACROPHAGES) | (ug/1E6 FLC) | (ug/ug FLC protein) |
|---|---|---|---|---|---|
| 0-GR | 2 | 7 | 103.8 | 100.1 | 1.62 |
| | 3 | 7 | 369.3 | 363.0 | 4.09 |
| 1-GR | 2 | 4 | 261.8 | 174.6 | 1.15 |
| | 3 | 7 | 227.3 | 180.5 | 1.44 |
| 2-GR | 3 | 4 | 236.5 | 211.2 | 1.16 |

BC TABLE 9

FLC PHOSPHOLIPID

Remarks: data calculated on the basis of inorganic phosphorus determination and mean phospholipid molecular weight of 775, calculated on the basis of various parameters

~~(a) treatment group versus control group~~
~~(b) relative molecular mass~~
~~(c) values not used to calculate mean quotient (see BC TABLE ...)~~

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SUBREPORT P 0500/3057 GD91 (R) A14 PS

| GROUP | EXPERIMENT NO. | FAME (~~TOTAL AREA 1E6/1E6 FLC~~) ← (U/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 5.76 | – | – | 3.~~94~~83 | 3.06 | 3.3~~1~~3 | 4.18 | 5.57 | 4.09 | 4.43 |
| | 2 | 5.57 | – | – | 3.79 | 2.7~~4~~5 | 3.0~~4~~5 | 3.7~~4~~3 | 5.1~~3~~4 | 3.26 | 3.87 |
| | 3 | 9.8~~5~~6 | 3.3~~8~~7 | 7.24 | 7.68 | – | – | 3.76 | 2.79 | 3.26 | 3.20 |
| 1-GR | 1 | 2.99 | – | – | ~~3.15~~ 1.73 | – | – | 1.96 | 2.6~~9~~8 | 2.4~~3~~2 | 2.37 |
| | 2 | 2.85 | – | – | – | 2.58 | – | 1.50 | – | 5.01 | – |
| | 3 | 2.9~~9~~8 | 2.05 | 2.40 | 1.64 | 2.78 | 2.32 | 1.36 | 2.2~~2~~1 | 2.90 | 2.33 |
| 2-GR | 3 | – | 4.5~~3~~2 | – | 3.43 | 3.33 | 3.37 | 4.7~~4~~3 | 3.24 | 3.81 | 3.76 |

BC TABLE 10

~~TOTAL AREA OF~~ FATTY ACID METHYL ESTERS FROM FLC

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2,3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

2029028816

10

SUBREPORT P 0500/3057 GD91 (R) A15 PS

| GROUP | STATISTICAL PARAMETER | FAME ~~(TOTAL AREA 1E6/1E6 FLC)~~ (U/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 1 | 1 | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| | M x̄ | 7.06 | 3.387 | 7.24 | 5.140 | 2.901 | 3.2119 | 3.89 | 4.50 | 3.54 | 3.83 |
| | SE | 1.40 | - | - | 1.279 | - | - | 0.145 | 0.86 | 0.28 | 0.36 |
| | RSD (0/0) | 34.3 | - | - | ~~42.9~~ 43.8 | - | - | 6.45 | 33.23 | 13.5 | 16.1 |
| 1-GR | N | 3 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 3 | 2 |
| | M x̄ | 2.94 | 2.05 | 2.40 | ~~2.40~~ 1.63 | 2.68 | 2.32 | 1.61 | 2.465 | 3.454 | 2.35 |
| | SE | 0.05 | - | - | - | - | - | 0.18 | - | 0.7980 | - |
| | RSD (0/0) | 2.7 | - | - | - | - | - | 19.5 | - | ~~39.9~~ 40.0 | - |
| 2-GR | x | - | 4.532 | - | 3.43 | 3.33 | 3.37 | 4.743 | 3.24 | 3.81 | 3.76 |

BC TABLE 11

TOTAL AREA OF FATTY ACID METHYL ESTERS FROM FLC, STATISTICAL PARAMETERS

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2, 2 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

2029028817

SUBREPORT P 0500/3057 GD91 (R) A16 PS

| GROUP | EXPERIMENT NO. | PALMITIC ACID METHYL ESTER (ug/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 68.7 | - | - | 41.9 | 21.4 | 28.5 | 49.3 | 35.8 | 23.9 | 36.3 |
| | 2 | 47.7 | - | - | 38.5 | 17.6 | 23.7 | 53.4 | 32.3 | 21.7 | 30.6 |
| | 3 | 37.5 | 30.6 | 34.7 | 43.3 | - | - | 38.0 | 19.6 | 19.0 | 22.4 |
| 1-GR | 1 | 40.7 | - | - | 17.3 | - | - | 25.6 | 22.6 | 18.5 | 21.3 |
| | 2 | 31.6 | - | - | - | 24.2 | - | 9.4 | - | 25.9 | - |
| | 3 | 20.6 | 18.9 | 19.5 | 18.3 | 33.0 | 27.1 | 22.0 | 20.7 | 26.8 | 23.9 |
| 2-GR | 3 | - | 25.9 | - | 31.7 | 26.9 | 28.6 | 40.1 | 25.1 | 37.3 | 33.9 |

BC TABLE 12

PALMITIC ACID METHYL ESTER (16:0) FROM FLC

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2, 2 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

SUBREPORT P 0500/3057 GD91 (R) A17 PS

| GROUP | STATISTICAL PARAMETER | PALMITIC ACID METHYL ESTER (ug/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 1 | 1 | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| | x̄ | 51.23 | 30.6 | 34.7 | 41.23 | 19.50 | 26.10 | 46.93 | 29.20 | 21.50 | 29.73 |
| | SE | 9.18 | - | - | 1.43 | - | - | 4.57 | 4.91 | 1.42 | 4.03 |
| | RSD (0/0) | 31.0 | - | - | 6.0 | - | - | 16.9 | 29.1 | 11.4 | 23.5 |
| 1-GR | N | 3 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 3 | 2 |
| | x̄ | 30.97 | 18.9 | 19.5 | 17.80 | 28.60 | 27.1 | 19.00 | 21.65 | 23.73 | 22.60 |
| | SE | 5.81 | - | - | - | - | - | 4.91 | - | 2.63 | - |
| | RSD (0/0) | 32.5 | - | - | - | - | - | 44.8 | - | 19.2 | - |
| 2-GR | x | - | 25.9 | - | 31.7 | 26.9 | 28.6 | 40.1 | 25.1 | 37.2 | 33.9 |

BC TABLE 13

PALMITIC ACID METHYL ESTER (16:0) FROM FLC, STATISTICAL PARAMETERS

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2, 2 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

2029028819

910

SUBREPORT P 0500/3057 GD91 (R) A18 PS

| GROUP | EXPERIMENT NO. | STEARIC ACID METHYL ESTER (ug/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 15.~~1~~0 | – | – | 11.5 | 10.1 | 10.6 | 12.6 | 13.~~7~~8 | 7.2 | 10.7 |
| | 2 | 12.5 | – | – | 11.5 | 8.~~3~~4 | 9.~~2~~3 | 12.3 | ~~14.0~~ 13.3 | 10.6 | 11.~~9~~8 |
| | 3 | 1.6 ~~11.5~~(a) | 10.~~1~~3(a) | – | ~~21.1~~ | – 12.1 | – | 11.6 | 9.~~4~~5 | 11.7 | 11.0 |
| 1-GR | 1 | 6.9 | – | – | 4.7 | – | – | 5.9 | 9.1 | 8.6 | 8.0 |
| | 2 | 5.~~6~~7 | – | – | – | 7.8 | – | 2.4 | – | 8.~~6~~5 | – |
| | 3 | 5.6 | 6.6 | 6.2 | 4.8 | 8.5 | 7.0 | 3.5 | 5.8 | 9.~~3~~2 | 6.9 |
| 2-GR | 3 | – | 11.6 | – | 10.0 | ~~10.9~~ 11.0 | 10.6 | 8.8 | 10.0 | 12.~~4~~3 | 11.~~2~~1 |

BC TABLE 14

STEARIC ACID METHYL ESTER (18:0) FROM FLC

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2, 2 (3) and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

(a) outlier not used in the calculation of means

2029028820

SUBREPORT P 0500/3057 GD91 (R) A19 PS

| GROUP | STATISTICAL PARAMETER | STEARIC ACID METHYL ESTER (ug/1E6 FLC) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 2 | - | - | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| | M x̄ | 13.8075 | - | - | 11.70 | 9.205 | 9.905 | 12.[illegible]7 | 12.3740 | 9.83 | 11.2017 |
| | SE | - | - | - | 0.20 | - | - | 0.30 | 1.495 | 1.35 | 0.363 |
| | RSD (0/0) | - | - | - | 3.0 | - | - | 4.2 | 20.83 | 23.9 | 5.01 |
| 1-GR | N | 3 | 1 | 1 | 2 | 2 | 1 | 3 | 2 | 3 | 2 |
| | M x̄ | 6.037 | 6.60 | 6.20 | 4.75 | 8.15 | 7.0 | 3.93 | 7.45 | 8.8377 | 7.45 |
| | SE | 0.432 | - | - | - | - | - | 1.03 | - | 0.232 | - |
| | RSD (0/0) | ~~12.4~~ 11.9 | - | - | - | - | - | 45.5 | - | 4.63 | - |
| 2-GR | x | - | 11.60 | - | 10.00 | ~~10.9~~ 11.0 | 10.6 | 8.8 | 10.0 | 12.43 | 11.21 |

BC TABLE 15

STEARIC ACID METHYL ESTER (18:0) FROM FLC, STATISTICAL PARAMETERS

Remarks: 1 determination of a single pool, 5 rats per pool
Pool 1 and 2, 2 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of cells per pool.

3

2029028821

SUBREPORT P 0500/3057 GD91 (R) A20 PS

| GROUP | POOL NO. | FAME ~~(ug/1E6 FLC) RATIO~~ ((g/1E6 FLC)/(g/1E6 FLC)) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | EXPERIMENT | | | STATISTICAL PARAMETER | | | |
| | | 1 | 2 | 3 | N | M | SE | RSD (0/0) |
| 0-GR | 1 | 4.5~~4~~8 | 3.82 | # 23.44(a) | 2 | 4.~~18~~20 | - | - |
| | 2 | - | - | # 2.97(a) | - | - | - | - |
| | 1 and 2 | - | - | - | - | - | - | - |
| | 3 | 3.64 | 3.35 | 3.58 | 3 | 3.52 | 0.09 | 4.3 |
| | 4 | 2.12 | 2.1~~2~~0 | - | 2 | 2.1~~2~~1 | - | - |
| | 3 and 4 | 2.6~~7~~9 | 2.5~~8~~5 | - | 2 | 2.6~~3~~2 | - | - |
| | 5 | 3.91 | 4.34 | 3.28 | 3 | 3.84 | 0.31 | 13.9 |
| | 6 | 2.~~61~~59 | 2.3~~0~~2 | 2.0~~9~~6 | 3 | 2.3~~3~~2 | 0.15 | 11.~~2~~4 |
| | 7 | 3.32 | 2.0~~4~~5 | 1.62 | 3 | 2.33 | 0.51 | ~~38.4~~ 37.9 |
| | 5, 6 and 7 | 3.39 | 2.5~~6~~9 | 2.04 | 3 | 2.6~~6~~7 | 0.39 | 25.~~6~~4 |

BC TABLE 16

RATIO OF PALMITIC ACID METHYL ESTER (16:0) VERSUS STEARIC ACID METHYL ESTER (18:0)

→ Einzelwerte und Mittelwerte auf getrennten Tabellen siehe Tab. 14/15

(a) outlier not used in the calculation of means

2029028822

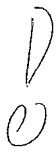
SUBREPORT P 0500/3057 GD91 (R) A21 PS

MD

| GROUP | POOL NO. | FAME (~~ug/1E6-FLC)~~ ~~RATIO~~ ((g/1E6 FLC)/(g/1E6 FLC)) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | EXPERIMENT | | | STATISTICAL PARAMETER | | | |
| | | 1 | 2 | 3 | N | M | SE | RSD (0/0) |
| 1-GR | 1 | 5.90 | 5.54 | 3.68 | 3 | 5.04 | 0.65 | 23.6 |
| | 2 | - | - | 2.86 | 1 | 2.86 | - | - |
| | 1 and 2 | - | - | 3.15 | 1 | 3.15 | - | - |
| | 3 | 3.68 | - | 3.81 | 2 | 3.75 | - | - |
| | 4 | - | 3.10 | 3.88 | 2 | 3.49 | - | - |
| | 3 and 4 | - | - | 3.87 | 1 | 3.87 | - | - |
| | 5 | 4.34 | 3.92 | 6.29 | 3 | 4.85 | 0.73 | 26.1 |
| | 6 | 2.48 | - | 3.57 | 2 | 3.03 | - | - |
| | 7 | 2.15 | 3.05 | 2.91 | 3 | 2.70 | 0.28 | 17.9 |
| | 5, 6 and 7 | 2.66 | - | 3.46 | 2 | 3.06 | - | - |

BC TABLE 16 (continued)

RATIO OF PALMITIC ACID METHYL ESTER (16:0) VERSUS STEARIC ACID METHYL ESTER (18:0)



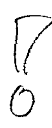
2029028823

Mo

| GROUP | POOL NO. | FAME ~~(ug/1E6 FLC)~~ ~~RATIO~~ ((g/1E6 FLC)/(g/1E6 FLC)) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | EXPERIMENT | | | STATISTICAL PARAMETER | | | |
| | | 1 | 2 | 3 | N | M | SE | RSD (0/0) |
| 2-GR | 1 | - | - | - | - | - | - | - |
| | 2 | - | - | 2.23 | 1 | 2.23 | - | - |
| | 1 and 2 | - | - | - | - | - | - | - |
| | 3 | - | - | 3.17 | 1 | 3.17 | - | - |
| | 4 | - | - | 2.4~~7~~5 | 1 | 2.4~~7~~5 | - | - |
| | 3 and 4 | - | - | 2.70 | 1 | 2.70 | - | - |
| | 5 | - | - | 4.56 | 1 | 4.56 | - | - |
| | 6 | - | - | 2.51 | 1 | 2.51 | - | - |
| | 7 | - | - | 3.0~~0~~3 | 1 | 3.0~~0~~3 | - | - |
| | 5, 6 and 7 | - | - | 3.0~~3~~5 | 1 | 3.0~~3~~5 | - | - |

BC TABLE 16 (continued)

RATIO OF PALMITIC ACID METHYL ESTER (16:0) VERSUS STEARIC ACID METHYL ESTER (18:0)



2029028824

SUBREPORT P 0500/3057 GD91 (R) A30 PS

| STATISTICAL PARAMETER | FAME ~~(ug/1E6 FLC)~~ ((g/1E6FLC)/(g/1E6FLC)) ~~MEAN~~ RATIO POOL 1, 3 ~~and~~ + 5 | POOL 2, 4, 6 ~~and~~ + 7 |
|---|---|---|
| N | 18 | 20 |
| M | 4.19 | 2.62 |
| SE | 0.21 | 0.13 |
| RSD (0/0) | 21.4 | 22.1 |

BC TABLE 17

MEAN RATIO PALMITIC ACID METHYL VERSUS STEARIC ACID METHYL ESTER

Remarks: all groups pooled

2029028825

SUBREPORT P 0500/3057 GD91 (R) A23 PS

no.

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 0-GR | 1 | 1 | 43.9 | | | 2 | ~~44.0~~ 42.9 | | | - | - | | |
| | | | 46.1 | | | | 42.4 | | | | - | | |
| | | | 46.4 | 45.47 | 3.0 | | 46.2 | ~~44.20~~ 43.83 | 4.3 7 | | - | - | - |
| | 2 | 1 | 60.9 | | | 2 | 53.2 | | | | - | | |
| | | | 61.0 | | | | 62.6 | | | | - | | |
| | | | 55.7 | 59.20 | 5.1 | | 58.0 | 53.93 (7) | 8.1 | - | - | - | - |
| | 3 | 1 | 223.6 | | | 2 | 374.2 | | | | - | | |
| | | | 220.8 | | | | 374.2 | | | | - | | |
| | | | 209.1 | 217.83 | 3.5 | | 377.0 | 375.13 | 0.4 | - | - | - | - |

BC TABLE 18

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028826

SUBREPORT P 0500/3057 GD91 (R) A24 PS

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 0-GR | 1 | 3 | 29.6 | | | 4 | 38.9 | | | - | - | | |
| | | | 31.5 | | | | 37.5 | | | | - | | |
| | | | 33.5 ~~32.2~~ | 31.53 ~~31.10~~ | 6.2 ~~4.3~~ | | 42.9 | 39.77 | 7.0 | | - | - | - |
| | 2 | 3 | 299.7 | | | 4 | 95.5 | | | - | - | | |
| | | | 311.6 | | | | 97.4 | | | | - | | |
| | | | 313.6 | 308.30 | 2.4 | | 95.9 | 96.27 | 1.0 | | - | - | - |
| | 3 | 3 | 395.7 | | | 4 | 776.7 | | | - | - | | |
| | | | 397.3 | | | | 831.7 | | | | - | | |
| | | | 412.0 | 401.67 | 2.24 | | 773.7 | 794.03 | 4.1 | | - | - | - |

BC TABLE 18 (continued)

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028827

SUBREPORT P 0500/3057 GD91 (R) A25 PS

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 0-GR | 1 | 5 | 53.0 | | | 6 | 25.0 | | | 7 | 24.3 | | |
| | | | 54.0 | | | | 22.4 | | | | 22.0 | | |
| | | | 57.7 | 54.90 | 4.5 | | 26.7 | 24.70 | 8.8 | | 23.9 | 23.40 | 5.3 |
| | 2 | 5 | 355.0 | | | 6 | 323.8 | | | 7 | 334.3 | | |
| | | | 342.1 | | | | 311.8 | | | | 341.3 | | |
| | | | 354.1 | 350.40 | 2.06 | | 311.8 | 315.80 | 2.2 | | 352.8 | 342.80 | 2.7 |
| | 3 | 5 | 854.7 | | | 6 | 712.2 | | | 7 | 249.3 | | |
| | | | 864.2 | | | | 749.4 | | | | 251.9 | | |
| | | | 829.5 | 849.47 | 2.11 | | 701.0 | 720.87 | 3.5 | | 253.4 | 251.53 | 0.8 |

BC TABLE 18 (continued)

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028828

SUBREPORT P 0500/3057 GD91 (R) A26 PS

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 1-GR | 1 | 1 | 2.~~8~~ 2 | | | 2 | 19.2 | | | – | – | | |
| | | | 2.6 ~~3.3~~ | | | | 19.7 | | | | – | | |
| | | | – | 2.40 ~~3.05~~ | – | | 19.1 | 19.33 | 1.7 | | – | – | – |
| | 2 | 1 | 322.7 | | | 2 | 398.7 | | | – | – | | |
| | | | 310.9 | | | | 338.1 | | | | – | | |
| | | | 268.8 | 300.80 | 9.4 | | 369.3 | 368.70 | 8.2 | | – | – | – |
| | 3 | 1 | 424.4 | | | 2 | 557.4 | | | – | – | | |
| | | | 410.5 | | | | 545.0 | | | | – | | |
| | | | 450.4 | 428.43 | 4.7 | | 553.2 | 551.87 | 1.1 | | – | – | – |

BC TABLE 19

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028829

SUBREPORT P 0500/3057 GD91 (R) A27 PS

MO.

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 1-GR | 1 | 3 | 5.9 | | | 4 | 26.0 | | | – | – | | |
| | | | 4.9 | | | | 26.5 | | | | – | | |
| | | | 5.1 | 5.30 | 10.0 | | 41.2 | 31.23 | 27.6 | | – | – | – |
| | 2 | 3 | – | | | 4 | 353.8 | | | – | – | | |
| | | | – | | | | 388.3 | | | | – | | |
| | | | – | – | – | | 352.3 | 364.80 | 5.6 | | – | – | – |
| | 3 | 3 | 254.9 | | | 4 | 903.4 | | | – | – | | |
| | | | 246.7 | | | | 989.9 ~~990.0~~ | | | | – | | |
| | | | 252.8 | 251.47 | 1.7 | | 1000.5 | 964.6~~3~~0 | 5.5 | | – | – | – |

BC TABLE 19 (continued)

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028830

SUBREPORT P 0500/3057 GD91 (R) A28 PS

| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) | POOL | x | M | RSD (0/0) |
| 1-GR | 1 | 5 | 37.1 | | | 6 | ~~39.6~~ 39.0 | | | 7 | 109.0 | | |
| | | | 34.9 | | | | 39.7 | | | | 103.9 | | |
| | | | 34.0 | 35.33 | 4.5 | | 39.7 | ~~39.5~~ 39.47 | 1.0 | | 98.6 | 103.83 | 5.0 |
| | 2 | 5 | 132.9 | | | 6 | - | | | 7 | 569.2 | | |
| | | | 129.8 | | | | - | | | | 548.2 | | |
| | | | 131.7 | 131.47 | 1.2 | | - | - | - | | 521.9 | 546.43 | 4.3 |
| | 3 | 5 | 419.0 | | | 6 | 501.1 | | | 7 | 489.5 | | |
| | | | 427.5 | | | | 495.5 | | | | 495.5 | | |
| | | | 383.8 ~~405.5~~ | 410.10 ~~417.33~~ | 5.6 ~~2.7~~ | | 503.1 | 499.90 | 0.8 | | 479.6 | 488.20 | 1.6 |

BC TABLE 19 (continued)

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

2029028831

SUBREPORT P 0500/3057 GD91 (R) A29 PS

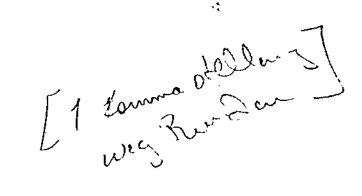
| GROUP | EXP. NO. | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) | | | |
|---|---|---|---|---|---|
| | | POOL | x | M | RSD (0/0) |
| 2-GR | 3 | 1 | 487.7 | | |
| | | | 505.3 | | |
| | | | 477.30 | 490.~~10~~ 00 | 2.9 |
| | | 2 | 554.1 | | |
| | | | 550.1 | | |
| | | | 569.8 | 558.00 | 1.9 |
| | | 3 | 709.9 | | |
| | | | 686.5 | | |
| | | | 664.0 | 686.80 | 3.3 |
| | | 4 | 428.5 | | |
| | | | 399.1 | | |
| | | | 370.4 | 399.33 | 7.3 |
| | | 5 | 766.0 | | |
| | | | 783.6 | | |
| | | | 810.9 | 786.83 | 2.9 |
| | | 6 | 589.9 | | |
| | | | 594.7 | | |
| | | | 575.4 | 586.67 | 1.7 |
| | | 7 | 332.0 | | |
| | | | 308.1 | | |
| | | | 324.0 | 321.37 | 3.8 |

BC TABLE 20

ACID PHOSPHATASE ACTIVITY

Remarks: M: the means of triplicate determination for each pool, 5 rats per pool

SUBREPORT P 0500/3057   GD91 (R) B1   PS



| GROUP | STATISTICAL PARAMETER | ACID PHOSPHATASE ACTIVITY (U/10E6 FLC) POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M | 107.500 | 159.087 | 247.023 | 310.023 | 418.2573 | 353.7908 | 205.910 |
| | SE | 55.307 | 108.0942 | 111.2751 | 242.562 | 231.8689 | 201.8639 | 94.982 |
| | RSD (0/0) | 89.1 | 117.79 | 78.0 | 135.5 | 96.0 | 98.8 | 79.9 |
| 1-GR | N | 3 | 3 | 2 | 3 | 3 | 2 | 3 |
| | M | 244.093 | 313.300 | 128.3854 | 453.553 | 194.710 | 269.700 | 379.4875 |
| | SE | 126.027 | 156.207 | - | 273.0791 | 114.718 | - | 138.850 |
| | RSD (0/0) | 89.4 | 86.4 | - | 104.3 | 102.0 | - | 63.4 |
| 2-GR | M | 490.X0 | 558.00 | 686.80 | 399.33 | 786.83 | 586.67 | 321.374 |
| | SE | - | - | - | - | - | - | - |
| | RSD (0/0) | - | - | - | - | - | - | - |

BC TABLE 21

Mean ACID PHOSPHATASE ACTIVITY, STATISTICAL PARAMETERS

~~Remarks: M: the means of 3 pools, 1 triplicate determination for each pool, 5 rats per pool~~

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| GROUP | POOL NO. | STATISTICAL PARAMETER | ACID PHOSPHATASE (U/1E6 FLC) ~~MEAN~~ RATIO (a) EXPERIMENT 1 | 2 | 3 | STATISTICAL PARAMETER M | SE | RSD (0/0) |
|---|---|---|---|---|---|---|---|---|
| 1-GR | 1 | | 0.1 | 5.1 | 2.0 | 2.40 | 1.46 | 105.2 |
| | 2 | | 0.4 | 6.4 | 1.5 | 2.77 | 1.84 | 115.~~2~~5 |
| | 3 | | 0.2 | - | 0.6 | 0.40 | - | - |
| | 4 | | 0.8 | 3.8 | 1.2 | 1.93 | 0.94 | 84.3 |
| | 5 | | 0.6 | 0.4 | 0.5 | 0.50 | 0.06 | 20.0 |
| | 6 | | 1.6 | - | 0.7 | 1.15 | - | - |
| | 7 | | 4.4 | 1.6 | 1.9 | 2.63 | 0.89 | 58.4 |
| | | N | 7 | ~~7~~5 | 7 | 7 | ~~7~~5 | ~~7~~5 |
| | | M | 1.16 | 3.46 | 1.20 | 1.9~~4~~68 | ~~0.76~~1.04 | ~~67.8~~76.7 |
| | | SE | 0.57 | 0.10 | 0.24 | - | - | - |
| | | RSD (0/0) | 130.9 | 71.2 | 51.8 | - | - | - |
| 2-GR | 1 | | - | - | 2.~~3~~2 | 2.~~3~~2 | - | - |
| | 2 | | - | - | 1.5 | 1.5 | - | - |
| | 3 | | - | - | 1.7 | 1.7 | - | - |
| | 4 | | - | - | 0.5 | 0.5 | - | - |
| | 5 | | - | - | 0.9 | 0.9 | - | - |
| | 6 | | - | - | 0.8 | 0.8 | - | - |
| | 7 | | - | - | 1.3 | 1.3 | - | - |
| | | N | - | - | 7 | 7 | - | - |
| | | M | - | - | 1.2~~0~~7 | 1.2~~0~~7 | - | - |
| | | SE | - | - | 0.2~~3~~2 | - | - | - |
| | | RSD (0/0) | - | - | ~~47.6~~ 46.0 | - | - | - |

2 Tabellen

BC TABLE 22

RATIO OF ACID PHOSPHATASE ACTIVITY IN TREATMENT VERSUS CONTROL GROUP, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

(a) treatment group versus control group

SUBREPORT P 0500/3057 GD91 (R) B4 PS

| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF MACROPHAGES (0/0) POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 98.5 | 98.6 | 99.2 | 99.2 | 98.9 | 98.8 | 99.4 |
| | 2 | 99.2 | 97.2 | 98.4 | 98.3 | 96.5 | 94.7 | 96.4 |
| | 3 | 96.6 | 96.7 | 97.3 | 99.4 | 94.9 | 97.8 | 98.3 |
| 1-GR | 1 | 23.8 | 52.8 | 43.3 | 65.7 | 39.0 | 61.4 | 80.5 |
| | 2 | 28.7 | 54.2 | 30.3 | 66.7 | 23.2 | 46.6 | 66.9 |
| | 3 | 28.5 | 58.1 | 28.7 | 64.8 | 27.9 | 56.1 | 79.4 |
| 2-GR | 3 | 86.5 | 91.5 | 68.7 | 89.3 | 77.4 | 89.8 | 97.5 |

BC TABLE 23

RELATIVE NUMBER OF MACROPHAGES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

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SUBREPORT P 0500/3057 GD91 (R) B5 PS

| GROUP | STATISTICAL PARAMETER | RELATIVE NUMBER OF MACROPHAGES ~~ACID PHOSPHATASE ACTIVITY (u/10E6 FLC)~~ POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 98.10 | 97.50 | 98.30 | 98.97 | 96.77 | 97.10 | 98.03 |
| | SE | 0.78 | 0.57 | 0.55 | 0.34 | 1.16 | 1.23 | 0.88 |
| | RSD (0/0) | 1.4 | 1.0 | 1.0 | 0.6 | 2.1 | 2.2 | 1.5 |
| 1-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 27.00 | 55.03 | 34.10 | 65.73 | 30.03 | 54.70 | 75.60 |
| | SE | 1.60 | 1.59 | 4.62 | 0.55 | 4.68 | 4.33 | 4.36 |
| | RSD (0/0) | 10.3 | 5.0 | 23.5 | 1.4 | 27.0 | 13.7 | 10.0 |
| 2-GR | x (%) | 86.5 | 91.5 | 68.7 | 89.3 | 77.4 | 89.8 | 97.5 |

BC TABLE 24

~~MEAN~~ RELATIVE NUMBER OF MACROPHAGES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

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SUBREPORT P 0500/3057 GD91 (R) B6 PS

| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF MACROPHAGES (0/0) POOL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
| 0-GR | 1 | 99.2 | 99.8 | 99.6 | 96.7 | 97.3 | 97.1 | 97.0 | 98.8 | 97.5 | 97.6 |
| | 2 | 97.8 | 99.4 | 98.8 | 98.6 | 99.4 | 99.2 | 98.9 | 98.3 | 98.3 | 98.4 |
| | 3 | 97.5 | 99.3 | 98.2 | 97.4 | 99.6 | 99.3 | 96.4 | 99.1 | 98.9 | 98.5 |
| 1-GR | 1 | 41.3 | 79.5 | 67.7 | 37.1 | 72.1 | 53.1 | 45.4 | 74.0 | 92.3 | 76.1 |
| | 2 | 32.1 | 56.4 | 43.2 | - | 59.8 | - | 26.2 | 52.6 | 65.7 | 54.7 |
| | 3 | 46.3 | 52.0 | 49.9 | 37.2 | 67.3 | 55.2 | 24.8 | 45.7 | 73.5 | 53.8 |
| 2-GR | 3 | 93.8 | 91.3 | 92.2 | 71.1 | 90.3 | 83.5 | 72.0 | 90.4 | 92.4 | 89.0 |

BC TABLE 25

RELATIVE NUMBER OF MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500 cells
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

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SUBREPORT P 0500/3057 GD91 (R) B7 PS

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| GROUP | STATISTICAL PARAMETER | MEAN RELATIVE NUMBER OF MACROPHAGES POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 98.17 | 99.50 | 98.87 | 97.57 | 98.77 | 98.53 | 97.43 | 98.73 | 98.23 | 98.17 |
| | SE | 0.52 | 0.15 | 0.41 | 0.55 | 0.74 | 0.72 | 0.75 | 0.23 | 0.41 | 0.28 |
| | RSD (0/0) | 0.9 | 0.3 | 0.7 | 1.0 | 1.3 | 1.3 | 1.3 | 0.4 | 0.7 | 0.5 |
| 1-GR | N | 3 | 3 | 3 | ~~3~~ 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (0/0) | 39.90 | 62.63 | 53.60 | 37.15 | 66.40 | 54.15 | 32.13 | 57.43 | 77.17 | 61.53 |
| | SE | 4.16 | 8.53 | 7.31 | - | 3.58 | - | 6.65 | 8.52 | 7.89 | 7.29 |
| | RSD (0/0) | 18.1 | 23.6 | 23.6 | - | 9.3 | - | 35.8 | 25.7 | 17.7 | 20.5 |
| 2-GR | x ( % ) | 93.8 | 91.3 | 92.2 | 71.1 | 90.3 | 83.5 | 72.0 | 90.4 | 92.4 | 89.0 |

BC TABLE 26

MEAN RELATIVE NUMBER OF MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

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SUBREPORT P 0500/3057 GD91 (R) B8 PS

| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF GRANULOCYTES (0/0) POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 0.7 | 0.2 | 0.4 | 0.0 | 0.6 | 0.6 | 0.0 |
| | 2 | 0.0 | 0.0 | 0.8 | 0.4 | 2.7 | 3.8 | 3.0 |
| | 3 | 0.4 | 1.5 | 1.0 | 0.0 | 2.3 | 1.2 | 1.3 |
| 1-GR | 1 | 75.0 | 46.8 | 56.0 | 34.3 | 60.5 | 38.0 | 19.5 |
| | 2 | 71.0 | 45.6 | 69.2 | 33.1 | 76.8 | 53.0 | 33.0 |
| | 3 | 70.5 | 39.3 | 69.5 | 34.3 | 71.1 | 42.7 | 19.7 |
| 2-GR | 3 | 12.7 | 8.3 | 30.8 | 10.0 | 22.4 | 9.8 | 2.4 |

BC TABLE 27

RELATIVE NUMBER OF GRANULOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

SUBREPORT P 0500/3057   GD91 (R) B9   PS

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| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ RELATIVE NUMBER OF GRANULOCYTES POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.37 | 0.57 | 0.73 | 0.13 | 1.87 | 1.87 | 1.43 |
| | SE | 0.20 | 0.47 | 0.18 | 0.13 | 0.64 | 0.98 | 0.87 |
| | RSD (0/0) | 95.8 | 143.7 | 41.7 | 173.2 | 59.7 | 91.1 | 105.0 |
| 1-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 72.17 | 43.90 | 64.90 | 33.90 | 69.47 | 44.57 | 24.07 |
| | SE | 1.42 | 2.33 | 4.45 | 0.40 | 4.78 | 4.43 | 4.47 |
| | RSD (0/0) | 3.4 | 9.2 | 11.9 | 2.0 | 11.9 | 17.2 | 32.1 |
| 2-GR | x (%) | 12.7 | 8.3 | 30.8 | 10.0 | 22.4 | 9.8 | 2.4 |

BC TABLE 28

~~MEAN~~ RELATIVE NUMBER OF GRANULOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETER

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

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SUBREPORT P 0500/3057 GD91 (R) B10 PS

| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF GRANULOCYTES (0/0) POOL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
| 0-GR | 1 | 0.4 | 0.0 | 0.1 | 1.3 | 0.4 | 0.7 | 0.8 | 0.0 | 0.0 | 0.3 |
| | 2 | 1.2 | 0.2 | 0.6 | 1.0 | 0.6 | 0.7 | 1.1 | 1.7 | 1.7 | 1.6 |
| | 3 | 1.1 | 0.2 | 0.7 | 0.9 | 0.0 | 0.1 | 1.7 | 0.6 | 0.9 | 0.9 |
| 1-GR | 1 | 58.7 | 20.5 | 38.2 | 62.9 | 27.9 | 46.9 | 54.6 | 26.0 | 7.7 | 23.9 |
| | 2 | 67.6 | 43.6 | 56.6 | - | 39.5 | - | 73.3 | 47.2 | 33.9 | 44.9 |
| | 3 | 52.1 | 47.0 | 48.9 | 61.9 | 32.5 | 44.4 | 74.6 | 53.7 | 26.3 | 45.8 |
| 2-GR | 3 | 5.5 | 8.5 | 7.4 | 28.3 | 8.8 | 15.7 | 27.2 | 9.1 | 7.0 | 10.4 |

BC TABLE 29

RELATIVE NUMBER OF GRANULOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

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SUBREPORT P 0500/3057 GD91 (R) B11 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ RELATIVE NUMBER OF GRANULOCYTES POOL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.90 | 0.13 | 0.~~35~~47 | 1.07 | 0.33 | 0.50 | 1.20 | 0.77 | 0.87 | 0.93 |
| | SE | 0.25 | 0.07 | 0.~~25~~19 | 0.12 | 0.18 | 0.20 | 0.26 | 0.50 | 0.49 | 0.38 |
| | RSD (0/0) | 48.4 | 86.6 | ~~101.0~~ 68.9 | 19.5 | 91.7 | 69.3 | 38.2 | 112.5 | 98.1 | 69.7 |
| 1-GR | N | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (0/0) | 59.47 | 37.03 | 47.90 | 62.40 | 33.30 | ~~40.8~~ 45.65 | 67.50 | 42.30 | 22.63 | 38.20 |
| | SE | 4.49 | 8.32 | 5.34 | - | 3.37 | - | 6.46 | 8.36 | 7.78 | 7.15 |
| | RSD (0/0) | 13.1 | 38.9 | 19.3 | - | 17.5 | - | 16.6 | 34.2 | 59.6 | 32.4 |
| 2-GR | x ( % ) | 5.5 | 8.5 | 7.4 | 28.3 | 8.8 | 15.7 | 27.2 | 9.1 | 7.0 | 10.4 |

BC TABLE 30

~~MEAN~~ RELATIVE NUMBER OF GRANULOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

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SUBREPORT P 0500/3057 GD91 (R) B13 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ RELATIVE NUMBER OF LYMPHOCYTES POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 1.50 | 1.93 | 0.97 | 0.90 | 1.37 | 1.03 | 0.53 |
| | SE | 0.75 | 0.47 | 0.38 | 0.21 | 0.67 | 0.26 | 0.07 |
| | RSD (0/0) | 86.7 | 41.8 | 68.9 | 40.1 | 84.8 | 43.6 | 21.7 |
| 1-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.87 | 1.07 | 1.00 | 0.40 | 0.50 | 0.73 | 0.40 |
| | SE | 0.24 | 0.77 | 0.35 | 0.31 | 0.29 | 0.24 | 0.31 |
| | RSD (0/0) | 48.0 | 124.8 | 60.8 | 132.3 | 100.0 | 56.8 | 132.3 |
| 2-GR | x (%) | 0.8 | 0.2 | 0.5 | 0.8 | 0.2 | 0.4 | 0.1 |

BC TABLE 32

~~MEAN~~ RELATIVE NUMBER OF LYMPHOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

SUBREPORT P 0500/3057 GD91 (R) B14 PS

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| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF LYMPHOCYTES (0/0) POOL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
| 0-GR | 1 | 0.4 | 0.2 | 0.3 | 1.9 | 2.3 | 2.2 | 2.2 | 1.2 | 2.5 | 2.1 |
| | 2 | 1.0 | 0.4 | 0.6 | 0.4 | 0.0 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 3 | 1.4 | 0.6 | 1.1 | 1.6 | 0.4 | 0.6 | 1.9 | 0.4 | 0.2 | 0.5 |
| 1-GR | 1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 2 | 0.3 | 0.0 | 0.2 | - | 0.7 | - | 0.6 | 0.2 | 0.4 | 0.4 |
| | 3 | 1.7 | 1.0 | 1.3 | 0.9 | 0.2 | 0.5 | 0.6 | 0.7 | 0.2 | 0.4 |
| 2-GR | 3 | 0.8 | 0.2 | 0.4 | 0.6 | 0.9 | 0.8 | 0.8 | 0.6 | 0.6 | 0.6 |

BC TABLE 33

RELATIVE NUMBER OF LYMPHOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

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SUBREPORT P 0500/3057 GD91 (R) B12 PS

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| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF LYMPHOCYTES (0/0) POOL 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 0.7 | 1.2 | 0.4 | 0.8 | 0.6 | 0.6 | 0.6 |
| | 2 | 0.8 | 2.8 | 0.8 | 1.3 | 0.8 | 1.5 | 0.6 |
| | 3 | 3.0 | 1.8 | 1.7 | 0.6 | 2.7 | 1.0 | 0.4 |
| 1-GR | 1 | 1.2 | 0.4 | 0.7 | 0.0 | 0.5 | 0.6 | 0.0 |
| | 2 | 0.4 | 0.2 | 0.6 | 0.2 | 0.0 | 0.4 | 0.2 |
| | 3 | 1.0 | 2.6 | 1.7 | 1.0 | 1.0 | 1.2 | 1.0 |
| 2-GR | 3 | 0.8 | 0.2 | 0.5 | 0.8 | 0.2 | 0.4 | 0.1 |

BC TABLE 31

RELATIVE NUMBER OF LYMPHOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500

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SUBREPORT P 0500/3057 GD91 (R) B15 PS

| GROUP | STATISTICAL PARAMETER | MEAN RELATIVE NUMBER OF LYMPHOCYTES POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.93 | 0.40 | 0.67 | 1.30 | 0.90 | 0.97 | 1.37 | 0.53 | 0.90 | 0.87 |
| | SE | 0.29 | 0.12 | 0.23 | 0.46 | 0.71 | 0.63 | 0.69 | 0.35 | 0.80 | 0.63 |
| | RSD (0/0) | 53.9 | 50.0 | 60.6 | 61.1 | 136.5 | 113.5 | 87.3 | 114.6 | 154.4 | 126.6 |
| 1-GR | N | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.67 | 0.33 | 0.50 | 0.45 | 0.30 | 0.25 | 0.40 | 0.30 | 0.20 | 0.27 |
| | SE | 0.52 | 0.33 | 0.40 | - | 0.21 | - | 0.20 | 0.21 | 0.12 | 0.13 |
| | RSD (0/0) | 136.1 | 173.2 | 140.0 | - | 120.2 | - | 86.6 | 120.2 | 100 | 86.6 |
| 2-GR | x ( % ) | 0.8 | 0.2 | 0.4 | 0.6 | 0.9 | 0.8 | 0.8 | 0.6 | 0.6 | 0.6 |

BC TABLE 34

MEAN RELATIVE NUMBER OF LYMPHOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: sum of macrophages, granulocytes and lymphocytes taken to be 100 percent
total count: approx. 500
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the means of these pools weighted according to the number of cells per pool.

SUBREPORT P 0500/3057 GD91 (R) B16 PS

| GROUP | EXPERIMENT NO. | NUMBER OF MACROPHAGES/~~RAT (1E6)~~ (1E6/RAT) POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 0.59 | 1.00 | 1.59 | 0.77 | 1.45 | 2.22 | 1.21 | 0.65 | 1.24 | 3.10 |
| | 2 | 0.66 | 1.11 | 1.77 | 0.77 | 1.90 | 2.67 | 0.67 | 1.00 | 1.90 | 3.57 |
| | 3 | 0.45 | 0.31 | 0.76 | 0.23 | 1.28 | 1.51 | 0.53 | 1.01 | 1.71 | 3.25 |
| 1-GR | 1 | 0.31 | 0.69 | 1.00 | 0.70 | 1.15 | 1.85 | 0.50 | 0.77 | 2.04 | 3.31 |
| | 2 | 0.44 | 0.65 | 1.09 | 0.54 | 1.71 | 2.25 | 0.17 | 0.38 | 1.20 | 1.75 |
| | 3 | 0.29 | 0.54 | 0.83 | 0.62 | 1.66 | 2.28 | 0.40 | 0.79 | 2.26 | 3.45 |
| 2-GR | 3 | 0.16 | 0.28 | 0.44 | 0.79 | 1.83 | 2.62 | 0.25 | 0.71 | 1.31 | 2.27 |

BC TABLE 35

~~RELATIVE~~ NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER

Remarks: macrophages counted after centrifugation and resuspension

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SUBREPORT P 0500/3057 GD91 (R) B17 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ NUMBER OF MACROPHAGES POOL 1 | 2 | 1 + 2 | 3 | 4 | 3 + 4 | 5 | 6 | 7 | 5, 6 + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N (1EG/RAT) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (1EG) | 0.567 | 0.807 | 1.373 | 0.590 | 1.543 | 2.133 | 0.803 | 0.887 | 1.617 | 3.307 |
| | SE | 0.062 | 0.250 | 0.311 | 0.180 | 0.185 | 0.338 | 0.207 | 0.118 | 0.196 | 0.139 |
| | RSD (0/0) | 18.9 | 53.8 | 39.2 | 52.8 | 20.8 | 27.4 | 44.7 | 23.1 | 21.0 | 7.3 |
| 1-GR | N (1EG/RAT) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (1EG) | 0.347 | 0.627 | 0.973 | 0.620 | 1.507 | 2.127 | 0.357 | 0.647 | 1.833 | 2.837 |
| | SE | 0.047 | 0.045 | 0.076 | 0.046 | 0.179 | 0.139 | 0.098 | 0.134 | 0.323 | 0.545 |
| | RSD (0/0) | 23.5 | 12.4 | 13.6 | 12.9 | 20.6 | 11.3 | 47.4 | 35.7 | 30.5 | 33.3 |
| 2-GR | x (1EG/RAT) | 0.16 | 0.28 | 0.44 | 0.79 | 1.83 | 2.62 | 0.25 | 0.71 | 1.31 | 2.27 |

BC TABLE 36

~~MEAN~~ NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, STATISTICAL PARAMETERS

Remarks: macrophages counted after centrifugation and resuspension

2029028848

no.

SUBREPORT P 0500/3057 GD91 (R) B18 PS

| GROUP | EXPERIMENT NO. | NUMBER OF ~~MACROPHAGES/RAT (1E3)~~ GRANULOCYTES (1E3/RAT) POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 2 | 0 | 2 | 11 | 6 | 17 | 10 | 0 | 0 | 10 |
| | 2 | 8 | 2 | 10 | 8 | 11 | 19 | 8 | 18 | 33 | 59 |
| | 3 | 5 | 1 | 6 | 2 | 0 | 2 | 9 | 6 | 16 | 31 |
| 1-GR | 1 | 440 | 178 | 618 | 1189 | 446 | 1635 | 6003 | 271 | 171 | 1045 |
| | 2 | 927 | 503 | 1430 | - | 1130 | - | 476 | 341 | 619 | 1437 |
| | 3 | 326 | 489 | 815 | 1033 | 803 | 1836 | 1205 | 928 | 809 | 2942 |
| 2-GR | 3 | 9 | 26 | 36 | 314 | 178 | 492 | 94 | 71 | 99 | 265 |

BC TABLE 37

NUMBER OF GRANULOCYTES PER RAT, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: absolute number of granulocytes calculated on the basis of absolute and relative number of macrophages

2029028849

SUBREPORT P 0500/3057 GD91 (R) B19 PS

no.

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ NUMBER OF GRANULOCYTES/~~RAT (1E3)~~ POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (1E3/RAT) | 5.0 | 1.0 | 6.0 | 7.0 | 5.7 | 12.7 | 9.0 | 8.0 | 16.3 | 33.3 |
| | SE | 1.7 | 0.6 | 2.3 | 2.6 | 3.2 | 5.4 | 0.6 | 5.3 | 9.5 | 14.2 |
| | RSD (0/0) | 60.0 | 100.0 | 66.7 | 65.5 | 97.2 | 73.4 | 11.1 | 114.6 | 101.0 | 73.7 |
| 1-GR | N | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (1E3/RAT) | 564.3 | 390.0 | 954.3 | 1111.0 | 793.0 | 1735.5 | 76~~0.3~~1.3 | 513.3 | 533.0 | 1808.0 |
| | SE | 184.3 | 106.1 | 244.5 | - | 197.5 | - | 22~~5.2~~4.8 | 208.3 | 189.1 | 578.2 |
| | RSD (0/0) | 56.6 | 47.1 | 44.4 | - | 43.1 | - | 51.~~3~~2 | 70.3 | 61.5 | 55.4 |
| 2-GR | x (1E3/RAT) | 9 | 26 | 36 | 314 | 178 | 492 | 94 | 71 | 99 | 265 |

BC TABLE 38

~~MEAN~~ NUMBER OF GRANULOCYYTES PER RAT, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETERS

Remarks: absolute number of granulocytes calculated on the basis of absolute and relative number of macrophages

SUBREPORT P 0500/3057 GD91 (R) B20 PS

| GROUP | EXPERIMENT NO. | VIABILITY (0/0) POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 93.8 | 96.5 | 95.5 | 98.3 | 99.8 | 99.3 | 98.4 | 99.8 | 99.8 | 99.3 |
| | 2 | 98.7 | 98.8 | 98.8 | 99.4 | 100.0 | 99.8 | 98.4 | 99.4 | 99.6 | 99.3 |
| | 3 | 98.5 | 97.8 | 98.2 | 99.2 | 99.8 | 99.7 | 98.3 | 99.6 | 99.6 | 99.4 |
| 1-GR | 1 | 93.7 | 96.7 | 95.8 | 98.5 | 99.1 | 98.9 | 97.0 | 99.2 | 98.6 | 98.5 |
| | 2 | 97.9 | 95.9 | 96.7 | 98.8 | 99.4 | 99.3 | 97.4 | 99.2 | 99.4 | 99.2 |
| | 3 | 96.2 | 96.6 | 96.5 | 98.8 | 97.1 | 97.6 | 98.7 | 98.6 | 98.9 | 98.8 |
| 2-GR | 3 | 96.5 | 96.9 | 96.8 | 98.6 | 98.9 | 98.8 | 97.3 | 99.0 | 98.7 | 98.6 |

BC TABLE 39

VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM

Remarks: trypan blue method, approx. 500 macrophages counted in hemocytometer
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages per pool.

no.

SUBREPORT P 0500/3057 GD91 (R) B21 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ VIABILITY POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 97.00 | 97.70 | 97.50 | 98.97 | 99.87 | 99.60 | 98.37 | 99.60 | 99.67 | 99.33 |
| | SE | 1.60 | 0.67 | 1.01 | 0.34 | 0.07 | 0.15 | 0.03 | 0.12 | 0.07 | 0.03 |
| | RSD (0/0) | 2.9 | 1.2 | 1.80 | 0.6 | 0.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.1 |
| 1-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (0/0) | 95.93 | 96.40 | 96.33 | 98.70 | 98.53 | 98.60 | 97.70 | 99.00 | 98.97 | 98.83 |
| | SE | 1.22 | 0.25 | 0.27 | 0.10 | 0.72 | 0.51 | 0.51 | 0.20 | 0.23 | 0.20 |
| | RSD (0/0) | 2.2 | 0.5 | 0.5 | 0.2 | 1.3 | 0.9 | 0.9 | 0.3 | 0.4 | 0.4 |
| 2-GR | x (%) | 96.5 | 96.9 | 96.8 | 98.6 | 98.9 | 98.8 | 97.3 | 99.0 | 98.7 | 98.6 |

BC TABLE 40

~~MEAN~~ VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: trypan blue method, approx. 500 macrophages counted in hemocytometer
Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages per pool.

2029028852

SUBREPORT P 0500/3057 GD91 (R) B22 PS

| GROUP | EXPERIMENT NO. | NUMBER OF NONVIABLE MACROPHAGES/~~RAT (1E3)~~ (1E3/RAT) POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 37 | 35 | 72 | 13 | 3 | 16 | 19 | 1 | 2 | ~~22~~ 23 |
| | 2 | 9 | 13 | ~~21~~ 22 | 5 | 0 | 5 | 11 | 6 | 8 | ~~25~~ 24 |
| | 3 | 7 | 7 | 14 | 2 | 3 | ~~5~~ 4 | 9 | 4 | 7 | 20 |
| 1-GR | 1 | 20 | 23 | 42 | 11 | 10 | ~~20~~ 21 | 15 | 6 | 29 | 50 |
| | 2 | 9 | 27 | 36 | 6 | 10 | ~~16~~ 17 | 4 | 3 | 7 | ~~14~~ 15 |
| | 3 | 11 | 18 | 29 | 7 | 48 | ~~55~~ 56 | 5 | 11 | 25 | 41 |
| 2-GR | 3 | 6 | 9 | 14 | 11 | 20 | 31 | 7 | 7 | 17 | ~~32~~ 31 |

BC TABLE 41

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM

Remarks: absolute number of nonviable macrophages calculated on the basis of viability and absolute number of macrophages

SUBREPORT P 0500/3057 GD91 (R) B23 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ NUMBER OF NONVIABLE MACROPHAGES/~~RAT (1E3)~~ POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (1E3/RAT) | 17.7 | 18.3 | ~~35.7~~ 36.0 | 6.7 | 2.0 | 8.~~7~~3 | 13.0 | 3.7 | 5.7 | 22.3 |
| | SE | 9.7 | 8.5 | 18.~~3~~1 | 3.3 | 1.0 | 3.~~7~~8 | 3.1 | 1.5 | 1.9 | 1.~~8~~2 |
| | RSD (0/0) | 94.9 | 80.4 | ~~88.8~~ 87.3 | 85.3 | 86.6 | ~~73.3~~ 79.9 | 40.7 | 68.6 | 56.7 | ~~11.3~~ 9.3 |
| 1-GR | N | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | M (1E3/RAT) | 13.3 | 22.7 | 35.7 | 8.0 | 22.7 | ~~30.3~~ 31.3 | 8.0 | 6.7 | 20.3 | 35.~~0~~3 |
| | SE | 3.4 | 2.6 | 3.8 | 1.5 | 12.7 | 12.4 | 3.5 | 2.3 | 6.8 | 10.~~8~~5 |
| | RSD (0/0) | 44.0 | 19.9 | 18.2 | 33.1 | 96.8 | ~~70.7~~ 68.5 | 76.0 | 60.6 | 57.6 | ~~53.5~~ 51.4 |
| 2-GR | x (1E3/RAT) | 6 | 9 | 14 | 11 | 20 | 31 | 7 | 7 | 17 | ~~32~~ 31 |

BC TABLE 42

~~MEAN~~ NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: absolute number of nonviable macrophages calculated on the basis of viability and absolute number of macrophages

SUBREPORT P 0500/3057 GD91 (R) B24 PS

no.

| GROUP | EXPERIMENT NO. | RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES (0/0) POOL | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
| 0-GR | 1 | 1.17 | 1.56 | 1.42 | 0.60 | 1.40 | 1.12 | 0.61 | 1.18 | 1.21 | 0.97 |
| | 2 | 1.02 | 0.96 | 0.98 | 0.82 | – | – | 0.96 | 1.38 | 1.57 | 1.40 |
| | 3 | 0.49 | 2.98 | 1.51 | 0.56 | 0.75 | 0.72 | 0.39 | 1.33 | 1.13 | 1.07 |
| 1-GR | 1 | 2.30 | 6.19 | 4.98 | 7.53 | 4.65 | 5.74 | 6.28 | 4.43 | 7.16 | 6.39 |
| | 2 | 5.13 | 3.77 | 4.32 | – | 3.36 | – | 4.28 | 4.14 | 5.65 | 5.19 |
| | 3 | 2.96 | 7.22 | 5.73 | 7.23 | 7.72 | 7.59 | 6.36 | 2.50 | 2.18 | 2.74 |
| 2-GR | 3 | 3.01 | 2.91 | 2.95 | 2.24 | 3.51 | 3.13 | 1.35 | 1.85 | 2.59 | 2.22 |

BC TABLE 43

RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION

Remarks: Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages per pool.

2029028855

SUBREPORT P 0500/3057 GD91 (R) B25 PS

no.

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES POOL 1 | 2 | 1 and 2 | 3 | 4 | 3 and 4 | 5 | 6 | 7 | 5, 6 and 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| | M (0/0) | 0.893 | 1.833 | 1.303 | 0.660 | 1.075 | 0.920 | 0.653 | 1.297 | 1.303 | 1.147 |
| | SE | 0.206 | 0.599 | 0.164 | 0.081 | - | - | 0.166 | 0.060 | 0.135 | 0.130 |
| | RSD (0/0) | 40.0 | 56.6 | 21.8 | 21.2 | - | - | 44.0 | 8.0 | 18.0 | 19.6 |
| 1-GR | N | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (0/0) | 3.463 | 5.727 | 5.010 | 7.380 | 5.243 | 6.650 | 5.640 | 3.690 | 4.997 | 4.773 |
| | SE | 0.858 | 1.023 | 0.407 | - | 1.293 | - | 0.680 | 0.601 | 1.474 | 1.074 |
| | RSD (0/0) | 42.8 | 30.9 | 14.1 | - | 42.7 | - | 20.9 | 28.2 | 51.1 | 39.0 |
| 2-GR | x (%) | 3.01 | 2.91 | 2.95 | 2.24 | 3.51 | 3.13 | 1.35 | 1.85 | 2.59 | 2.22 |

BC TABLE 44

~~MEAN~~ RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, STATISTICAL PARAMETER

Remarks: Pool 1 and 2, 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages per pool.

2029028856

no.

SUBREPORT P 0500/3057 GD91 (R) B26 PS

| GROUP | EXPERIMENT NO. | NUMBER OF MULTINUCLEATED MACROPHAGES/~~[illegible]~~ (1E3/RAT) POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 1 | 6.9 | 15.6 (2.0) (a) | 22.5 | 4.6 | 20.3 | 24.9 | 7.4 | 7.6 | 15.0 | 30.0 |
| | 2 | 6.7 | 10.6 | 17.4 | 6.3 | - | - | 6.4 | 13.8 | 29.7 | 49.9 |
| | 3 | 2.2 | 9.2 | 11.4 | 1.3 | 9.6 | 10.9 | 2.1 | 13.4 (~~2.0~~ 1.9) (a) | 19.3 | 34.8 |
| 1-GR | 1 | 7.1 | 42.7 | 49.8 | 52.7 (7.5) (a) | 53.5 | 106.2 | 31.4 | 34.1 | 146.0 (4.2) (a) | 211.5 |
| | 2 | 22.6 | 24.5 | 47.1 | - | 57.5 | - | 7.3 | 15.7 | 67.7 (3.2) (a) | 90.7 |
| | 3 | 8.6 | 39.0 | 47.6 | 44.8 | 128.1 | 172.9 | 25.4 | 19.8 | 49.3 (4.9) (a) | 94.5 |
| 2-GR | 3 | 4.8 | 8.1 | 13.0 | 17.7 | 64.3 | 82.0 | 3.4 (0.7) (a) | 13.1 | 34.0 | 50.4 |

BC TABLE 45

NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM

Remarks: absolute number of multinucleated macrophages calculated from differential counts and absolute macrophage number

(a) number of macrophages with .GT.2 nuclei

2029028857

no.

SUBREPORT P 0500/3057 GD91 (R) B27 PS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ NUMBER OF MULTINUCLEATED MACROPHAGES (~~1E3~~) POOL 1 | 2 | 1 ~~and~~ + 2 | 3 | 4 | 3 ~~and~~ + 4 | 5 | 6 | 7 | 5, 6 ~~and~~ + 7 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 3 | 3 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 3 |
| | M (1E3) | 5.27 | 11.80 | 17.10 | 4.07 | ~~15.0~~ 14.55 | 17.90 | 5.30 | 11.60 | 21.33 | 38.23 |
| | SE | 1.53 | 1.94 | 3.21 | 1.47 | - | - | 1.63 | 2.00 | 4.36 | 6.00 |
| | RSD (0/0) | 50.5 | 28.5 | 32.5 | 62.5 | - | - | 53.1 | 29.9 | 35.4 | 27.2 |
| 1-GR | N | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 3 |
| | M (1E3) | 12.77 | 35.40 | 48.17 | 48.875 | 79.70 | 139.655 | 21.37 | 23.20 | 87.67 | 132.23 |
| | SE | 4.94 | 5.55 | 0.83 | - | 24.23 | - | 7.24 | 5.58 | 29.65 | 39.65 |
| | RSD (0/0) | 67.0 | 27.2 | 3.0 | - | 52.7 | - | 58.7 | 41.6 | 58.6 | 51.9 |
| 2-GR | x (1E3) | 4.8 | 8.1 | 13.0 | 17.7 | 64.3 | 82.0 | 3.4 | 13.1 | 34.0 | 50.4 |

BC TABLE 46

MEAN NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: absolute number of multinucleated macrophages calculated from differential counts and absolute macrophage number

2029028858

SUBREPORT P 0500/3057   GD103 (R) A1   WS

no -

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ MACROPHAGE AREA ~~(um2)~~ POOL 3 | 4 | 3 ~~AND~~ and 4 | 5 | 6 | 7 | 5, 6 ~~AND~~ and 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 107 | 133 | | 94 | 187 | 188 | |
| | M (μm²) | 204 | 211 | 210 | 232 | 214 | 227 | 224 |
| | SE | 6 | 5 | | 5 | 4 | 5 | |
| | RSD (0/0) | 28.7 | 28.3 | | 22.8 | 26.4 | 30.6 | |
| 1-GR | N | 88 | 77 | | 99 | 164 | 134 | |
| | M (μm²) | 335 | 376 | 365 | 306 | 274 | 267 | 273 |
| | SE | 13 | 15 | | 13 | 9 | 8 | |
| | RSD (0/0) | 37.2 | 35.8 | | 43.1 | 42.3 | 34.2 | |
| 2-GR | N | 100 | 90 | | 81 | 68 | 81 | |
| | M (μm²) | 242 | 267 | 259 | 201 | 262 | 297 | 275 |
| | SE | 8 | 10 | | 8 | 12 | 10 | |
| | RSD (0/0) | 34.4 | 35.2 | | 37.3 | 37.2 | 31.1 | |

BC TABLE 47

MEAN MACROPHAGE AREA, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: Pool 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages/pool.

2029028859

SUBREPORT P 0500/3057 GD103 (R) A2 . WS

| GROUP | POOL | DISTRIBUTION OF MACROPHAGES (0/0) AREA CLASS 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 3 | 0.0 | 0.0 | 1.9 | 1.9 | 24.3 | 37.4 | 28.0 | 6.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 4 | 0.0 | 0.0 | 0.0 | 2.3 | 18.8 | 45.9 | 24.8 | 6.8 | 1.5 | 0.0 | 0.0 | 0.0 |
| | 5 | 0.0 | 0.0 | 0.0 | 0.0 | 8.5 | 42.6 | 38.3 | 9.6 | 1.1 | 0.0 | 0.0 | 0.0 |
| | 6 | 0.0 | 0.0 | 0.0 | 0.5 | 19.8 | 42.2 | 29.4 | 7.5 | 0.5 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 1.6 | 15.4 | 35.6 | 33.0 | 12.2 | 1.6 | 0.5 | 0.0 | 0.0 |
| 1-GR | 3 | 0.0 | 0.0 | 0.0 | 1.1 | 3.4 | 12.5 | 26.1 | 38.6 | 10.2 | 6.8 | 1.1 | 0.0 |
| | 4 | 0.0 | 0.0 | 0.0 | 0.0 | 5.2 | 5.2 | 23.4 | 26.0 | 27.3 | 13.0 | 0.0 | 0.0 |
| | 5 | 0.0 | 0.0 | 0.0 | 1.0 | 5.1 | 24.2 | 28.3 | 24.2 | 11.1 | 5.1 | 1.0 | 0.0 |
| | 6 | 0.0 | 0.0 | 1.2 | 4.9 | 4.9 | 29.9 | 26.2 | 21.3 | 9.8 | 1.8 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 1.5 | 8.2 | 23.9 | 41.8 | 15.7 | 9.0 | 0.0 | 0.0 | 0.0 |
| 2-GR | 3 | 0.0 | 0.0 | 0.0 | 3.0 | 15.0 | 31.0 | 29.0 | 19.0 | 3.0 | 0.0 | 0.0 | 0.0 |
| | 4 | 0.0 | 0.0 | 0.0 | 2.2 | 12.2 | 20.0 | 33.3 | 25.6 | 5.6 | 1.1 | 0.0 | 0.0 |
| | 5 | 0.0 | 0.0 | 0.0 | 9.9 | 23.5 | 42.0 | 16.0 | 4.9 | 3.7 | 0.0 | 0.0 | 0.0 |
| | 6 | 0.0 | 0.0 | 0.0 | 4.4 | 5.9 | 27.9 | 33.8 | 20.6 | 5.9 | 1.5 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 19.8 | 38.3 | 29.6 | 8.6 | 1.2 | 0.0 | 0.0 |

BC TABLE 48

RELATIVE DISTRIBUTION OF MACROPHAGES ACCORDING TO AREA

Remarks: (for number of cells per determination see BC TABLE ..) classification on the basis of
10 equal steps on logarithmic scale
range: 50 to 1000 um2
classes 0 and 11 out of range

NO

SUBREPORT P 0500/3057 GD103 (R) A3 WS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ NUCLEUS AREA ~~(um2)~~ POOL 3 | 4 | 3 ~~AND~~ and 4 | 5 | 6 | 7 | 5, 6 ~~AND~~ and 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 107 | 134 | | 95 | 192 | 192 | |
| | M (µm²) | 60.6 | 64.7 | 64.1 | 64.7 | 64.7 | 65.7 | 65.2 |
| | SE | 1.1 | 0.8 | | 1.0 | 0.8 | 0.7 | |
| | RSD (0/0) | 19.0 | 14.7 | | 15.2 | 17.6 | 15.1 | |
| 1-GR | N | 94 | 78 | | 103 | 171 | 136 | |
| | M (µm²) | 62.2 | 68.2 | 66.6 | 60.7 | 58.1 | 60.8 | 60.2 |
| | SE | 1.3 | 1.3 | | 1.2 | 1.0 | 1.2 | |
| | RSD (0/0) | 20.3 | 17.2 | | 19.6 | 23.2 | 23.5 | |
| 2-GR | N | 100 | 90 | | 84 | 69 | 81 | |
| | M (µm²) | 61.8 | 66.2 | 64.9 | 60.5 | 63.6 | 70.7 | 67.4 |
| | SE | 1.4 | 1.2 | | 1.3 | 1.3 | 1.2 | |
| | RSD (0/0) | 22.7 | 17.6 | | 19.1 | 16.8 | ~~70.7~~ 15.4 | |

BC TABLE 49

MEAN NUCLEUS AREA, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: Pool 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages/pool.

2029028861

SUBREPORT P 0500/3057 GD103 (R) A4 WS

| GROUP | POOL | DISTRIBUTION OF NUCLEI (0/0) AREA CLASS 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 3 | 0.0 | 0.9 | 3.7 | 10.3 | 27.1 | 33.6 | 13.1 | 5.6 | 3.7 | 0.0 | 0.0 | 1.9 |
| | 4 | 0.0 | 0.7 | 0.7 | 1.5 | 20.1 | 31.3 | 28.4 | 9.7 | 5.2 | 0.7 | 0.7 | 0.7 |
| | 5 | 0.0 | 0.0 | 0.0 | 5.3 | 16.8 | 32.6 | 30.5 | 8.4 | 2.1 | 1.1 | 3.2 | 0.0 |
| | 6 | 1.0 | 0.0 | 0.5 | 4.7 | 21.9 | 25.0 | 28.1 | 13.0 | 1.6 | 1.0 | 1.6 | 1.6 |
| | 7 | 0.0 | 0.5 | 0.0 | 2.1 | 18.8 | 28.1 | 32.8 | 11.5 | 2.6 | 1.0 | 1.6 | 1.0 |
| 1-GR | 3 | 0.0 | 0.0 | 8.5 | 8.5 | 21.3 | 22.3 | 19.1 | 9.6 | 7.4 | 2.1 | 0.0 | 1.1 |
| | 4 | 0.0 | 0.0 | 0.0 | 2.6 | 15.4 | 29.5 | 19.2 | 14.1 | 10.3 | 5.1 | 2.6 | 1.3 |
| | 5 | 0.0 | 3.9 | 3.9 | 9.7 | 24.3 | 24.3 | 16.5 | 13.6 | 1.9 | 1.0 | 1.0 | 0.0 |
| | 6 | 1.2 | 2.3 | 13.5 | 14.6 | 17.5 | 24.6 | 13.5 | 5.8 | 5.3 | 1.2 | 0.0 | 0.6 |
| | 7 | 1.5 | 2.2 | 5.1 | 16.2 | 15.4 | 25.7 | 16.9 | 9.6 | 3.7 | 0.7 | 2.2 | 0.7 |
| 2-GR | 3 | 0.0 | 3.0 | 7.0 | 10.0 | 19.0 | 20.0 | 22.0 | 10.0 | 4.0 | 3.0 | 0.0 | 2.0 |
| | 4 | 0.0 | 0.0 | 2.2 | 8.9 | 15.6 | 20.0 | 24.4 | 17.8 | 3.3 | 6.7 | 1.1 | 0.0 |
| | 5 | 0.0 | 0.0 | 6.0 | 16.7 | 21.4 | 28.6 | 9.5 | 9.5 | 4.8 | 3.6 | 0.0 | 0.0 |
| | 6 | 0.0 | 1.4 | 0.0 | 11.6 | 18.8 | 20.3 | 26.1 | 15.9 | 5.8 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 2.5 | 11.1 | 16.0 | 25.9 | 24.7 | 12.3 | 4.9 | 1.2 | 1.2 |

BC TABLE 50

RELATIVE DISTRIBUTION OF NUCLEI ACCORDING TO AREA

Remarks: (for number of cells per determination see BC TABLE ..) classification on the basis of
10 equal steps on logarithmic scale
range: 30 to 100 um2
classes 0 and 11 out of range

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SUBREPORT P 0500/3057   GD103 (R) A5   WS

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ VACUOLE AREA ~~(um2)~~ POOL 3 | 4 | 3 and ~~AND~~ 4 | 5 | 6 | 7 | 5, 6 and ~~AND~~ 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 172 | 321 | | 384 | 653 | 396 | |
| | M (µm²) | 0.73 | 0.59 | 0.61 | 0.67 | 0.74 | 0.91 | 0.82 |
| | SE | 0.09 | 0.03 | | 0.03 | 0.03 | 0.11 | |
| | RSD (0/0) | 163.2 | 94.3 | | ~~110.7~~ 89.9 | 110.7 | 230.7 | |
| 1-GR | N | 392 | 646 | | 997 | 1228 | 615 | |
| | M (µm²) | 3.35 | 1.78 | 2.21 | 2.51 | 2.51 | 2.18 | 2.29 |
| | SE | 0.24 | 0.12 | | 0.32 | 0.24 | 0.16 | |
| | RSD (0/0) | 141.8 | 167.3 | | 401.7 | 330.8 | 181.1 | |
| 2-GR | N | 553 | 524 | | 315 | 391 | 488 | |
| | M (µm²) | 0.99 | 1.04 | 1.02 | 1.18 | 0.90 | 0.97 | 0.97 |
| | SE | 0.12 | 0.07 | | 0.10 | 0.10 | 0.06 | |
| | RSD (0/0) | 275.4 | 161.5 | | 155.0 | 213.5 | 136.5 | |

BC TABLE 51

MEAN VACUOLE AREA, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: Pool 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages/pool.

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SUBREPORT P 0500/3057   GD103 (R) A6   WS

NO.

| GROUP | STATISTICAL PARAMETER | ~~MEAN~~ VACUOLE AREA PER ~~CELL~~ MACROPHAGE ~~(0/0)~~ POOL 3 | 4 | 3 ~~AND~~ and 4 | 5 | 6 | 7 | 5, 6 ~~AND~~ and 7 |
|---|---|---|---|---|---|---|---|---|
| 0-GR | N | 172 | 321 | – | 384 | 653 | 396 | – |
| | M (%) | 0.53 | 0.63 | 0.61 | 1.08 | 1.13 | 0.67 | 0.88 |
| | SE | – | – | – | – | – | – | |
| | RSD (0/0) | – | – | – | – | – | – | |
| 1-GR | N | 392 | 646 | – | 997 | 1228 | 615 | |
| | M (%) | 4.01 | 3.74 | 3.81 | 6.36 | 5.72 | 3.21 | 4.15 |
| | SE | – | – | – | – | – | – | |
| | RSD (0/0) | – | – | – | – | – | – | |
| 2-GR | N | 553 | 524 | – | 315 | 391 | 488 | |
| | M (%) | 1.97 | 1.96 | 1.96 | 2.14 | 1.69 | 1.73 | 1.76 |
| | SE | – | – | – | – | – | – | |
| | RSD (0/0) | – | – | – | – | – | – | |

BC TABLE 52

~~MEAN~~ VACUOLE AREA PER ~~CELL~~ MACROPHAGE, RESUSPENSION MEDIUM, STATISTICAL PARAMETERS

Remarks: Pool 3 and 4, 5, 6 and 7 represents the mean of these pools weighted according to the number of macrophages/pool.

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SUBREPORT P 0500/3057 GD103 (R) A7 WS

| GROUP | POOL | DISTRIBUTION OF VACUOLES (0/0) AREA CLASS 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0-GR | 3 | 0.0 | 6.4 | 29.7 | 37.8 | 14.5 | 7.0 | 2.9 | 0.6 | 1.2 | 0.0 | 0.0 | 0.0 |
| | 4 | 0.0 | 1.9 | 25.2 | 47.0 | 20.2 | 2.5 | 2.8 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 5 | 0.0 | 7.6 | 25.8 | 30.2 | 24.5 | 9.4 | 2.3 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 6 | 0.0 | 2.1 | 23.3 | 39.1 | 21.3 | 10.7 | 2.9 | 0.5 | 0.2 | 0.0 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.3 | 15.4 | 41.9 | 25.0 | 13.1 | 3.0 | 0.8 | 0.3 | 0.0 | 0.3 | 0.0 |
| 1-GR | 3 | 0.0 | 0.3 | 3.3 | 13.5 | 23.0 | 24.5 | 13.3 | 9.7 | 8.4 | 3.3 | 0.8 | 0.0 |
| | 4 | 0.0 | 0.5 | 13.5 | 29.7 | 23.5 | 14.7 | 8.8 | 4.3 | 3.9 | 1.1 | 0.0 | 0.0 |
| | 5 | 0.0 | 0.6 | 9.7 | 25.8 | 24.6 | 17.9 | 10.0 | 6.0 | 3.7 | 0.7 | 0.7 | 0.3 |
| | 6 | 0.0 | 0.5 | 7.0 | 20.8 | 24.8 | 22.5 | 11.4 | 7.8 | 3.3 | 1.3 | 0.5 | 0.2 |
| | 7 | 0.0 | 0.2 | 7.8 | 21.1 | 25.7 | 22.9 | 11.1 | 6.2 | 2.8 | 1.8 | 0.5 | 0.0 |
| 2-GR | 3 | 0.0 | 1.4 | 21.3 | 37.4 | 25.0 | 9.2 | 3.4 | 1.4 | 0.2 | 0.2 | 0.2 | 0.2 |
| | 4 | 0.0 | 3.4 | 21.4 | 25.6 | 26.9 | 14.5 | 5.5 | 1.7 | 0.6 | 0.4 | 0.0 | 0.0 |
| | 5 | 0.0 | 1.3 | 16.5 | 34.2 | 19.0 | 18.1 | 6.7 | 2.5 | 1.0 | 0.6 | 0.0 | 0.0 |
| | 6 | 0.0 | 3.6 | 19.2 | 35.8 | 23.8 | 13.3 | 3.6 | 0.0 | 0.5 | 0.0 | 0.3 | 0.0 |
| | 7 | 0.0 | 2.9 | 16.0 | 35.5 | 25.4 | 12.7 | 5.7 | 1.2 | 0.4 | 0.2 | 0.0 | 0.0 |

BC TABLE 53

DISTRIBUTION OF VACUOLES ACCORDING TO SIZE

Remarks: (for number of cells per determination see BC TABLE ..) classification on the basis of
10 equal steps on logarithmic scale
range: 0.1 to 50 um2
classes 0 and 11 out of range

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SUBREPORT P 0500/3057 GD151 (R) B15 WS M

BC FIGURE 1

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 0-GR

Remarks: A: pool 1, B: pool 2
representative field, resuspension medium, experiment 1

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BC FIGURE 2

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 1-GR

Remarks: A: pool 1, B: pool 2
representative field, resuspension medium, experiment 1

SUBREPORT P 0500/3057 GD151 (R) B15 WS M

BC FIGURE 3

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 0-GR

Remarks: A: pool 3, B: pool 4
representative field, resuspension medium, experiment 3

SUBREPORT P 0500/3057 GD151 (R) B15 WS M

BC FIGURE 4

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 0-GR

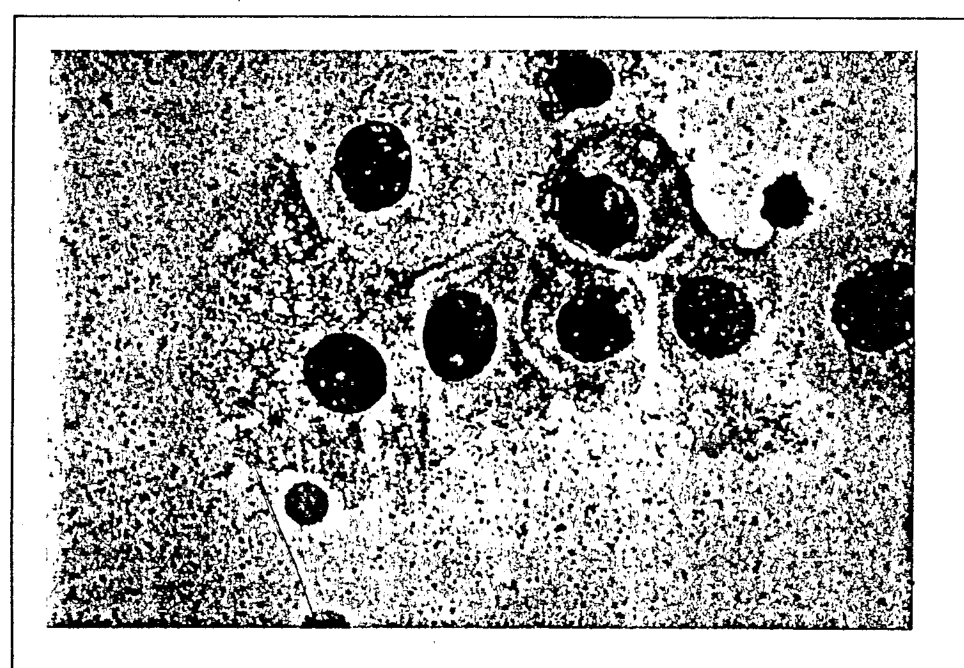
Remarks: A: pool 5, B: pool 6, C: pool 7
representative field, resuspension medium, experiment 3

A -13057

Datum :

ZN :

Blatt :



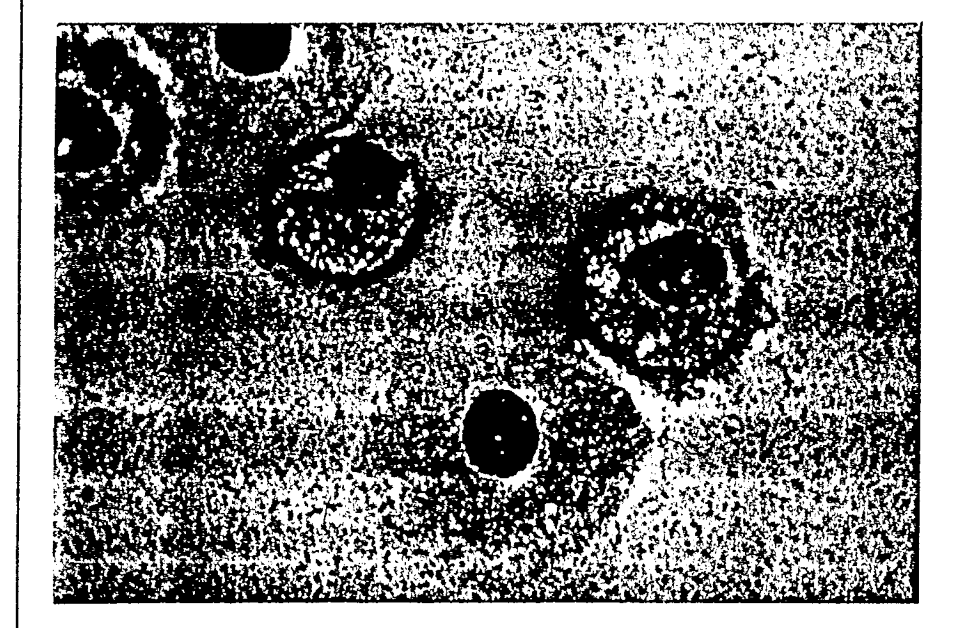


Figure 1

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Datum :

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Figure 2

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Datum :

ZN :

Blatt :

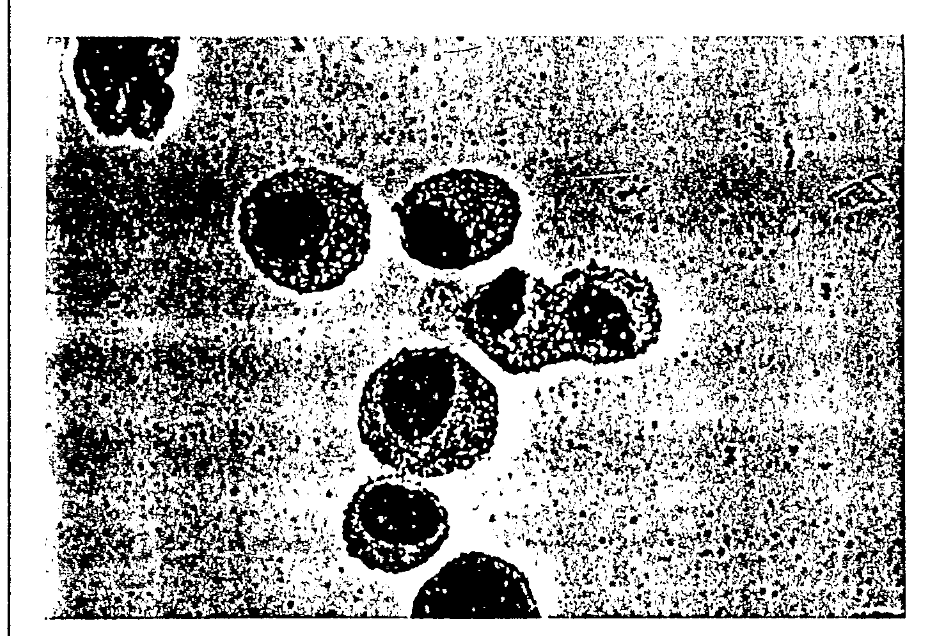


Figure 3

3057



Figure 4

A

B

C

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SUBREPORT P 0500/3057 GD151 (R) B16 WS M

BC FIGURE 5

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 1-GR

Remarks: A: pool 3, B: pool 4
representative field, resuspension medium, experiment 3

SUBREPORT P 0500/3057 GD151 (R) B16 WS M

BC FIGURE 6

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 1-GR

Remarks: A: pool 5, B: pool 6, C: pool 7
representative field, resuspension medium, experiment 3

SUBREPORT P 0500/3057 GD151 (R) B16 WS M

BC FIGURE 7

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 2-GR

Remarks: A: pool 3, B: pool 4
representative field, resuspension medium, experiment 3

SUBREPORT P 0500/3057 GD151 (R) B16 WS M

BC FIGURE 8

FREE LUNG CELLS IN CYTOCENTRIFUGE PREPARATIONS, 0-GR

Remarks: A: pool 5, B: pool 6, C: pool 7
representative field, resuspension medium, experiment 3

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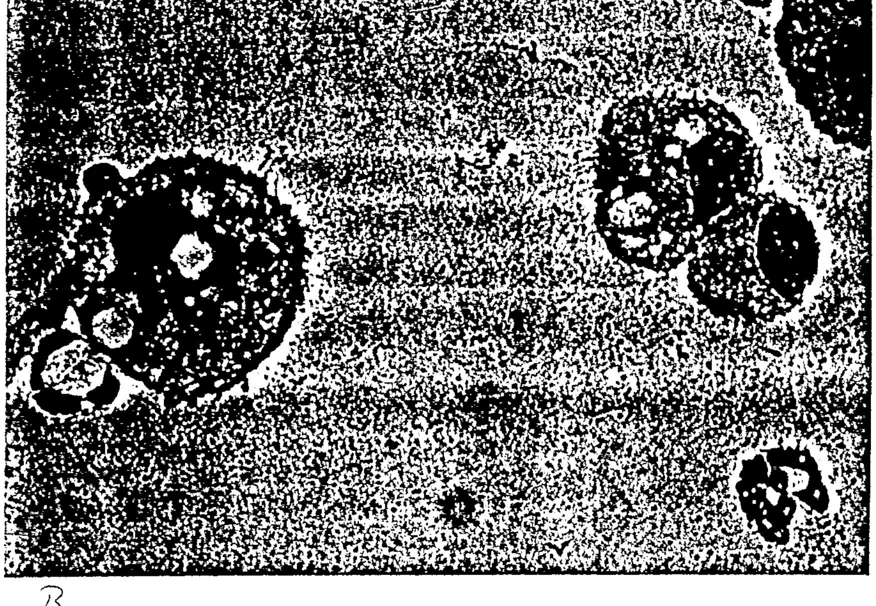
Datum :

ZN :

Blatt :



A



B

Figure 5

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.1-6R

Figure 6

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Datum :

ZN :

Blatt :

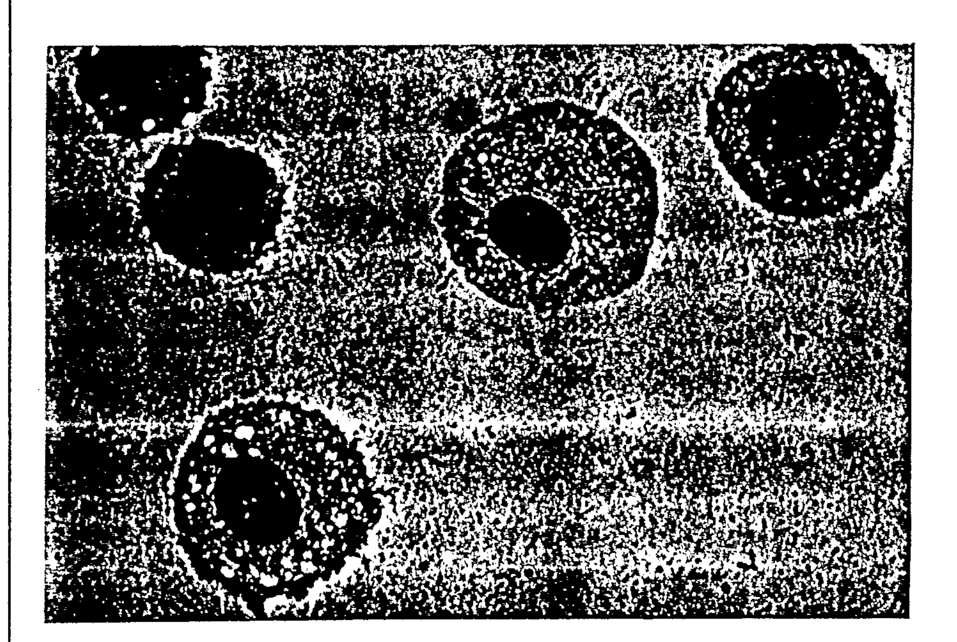


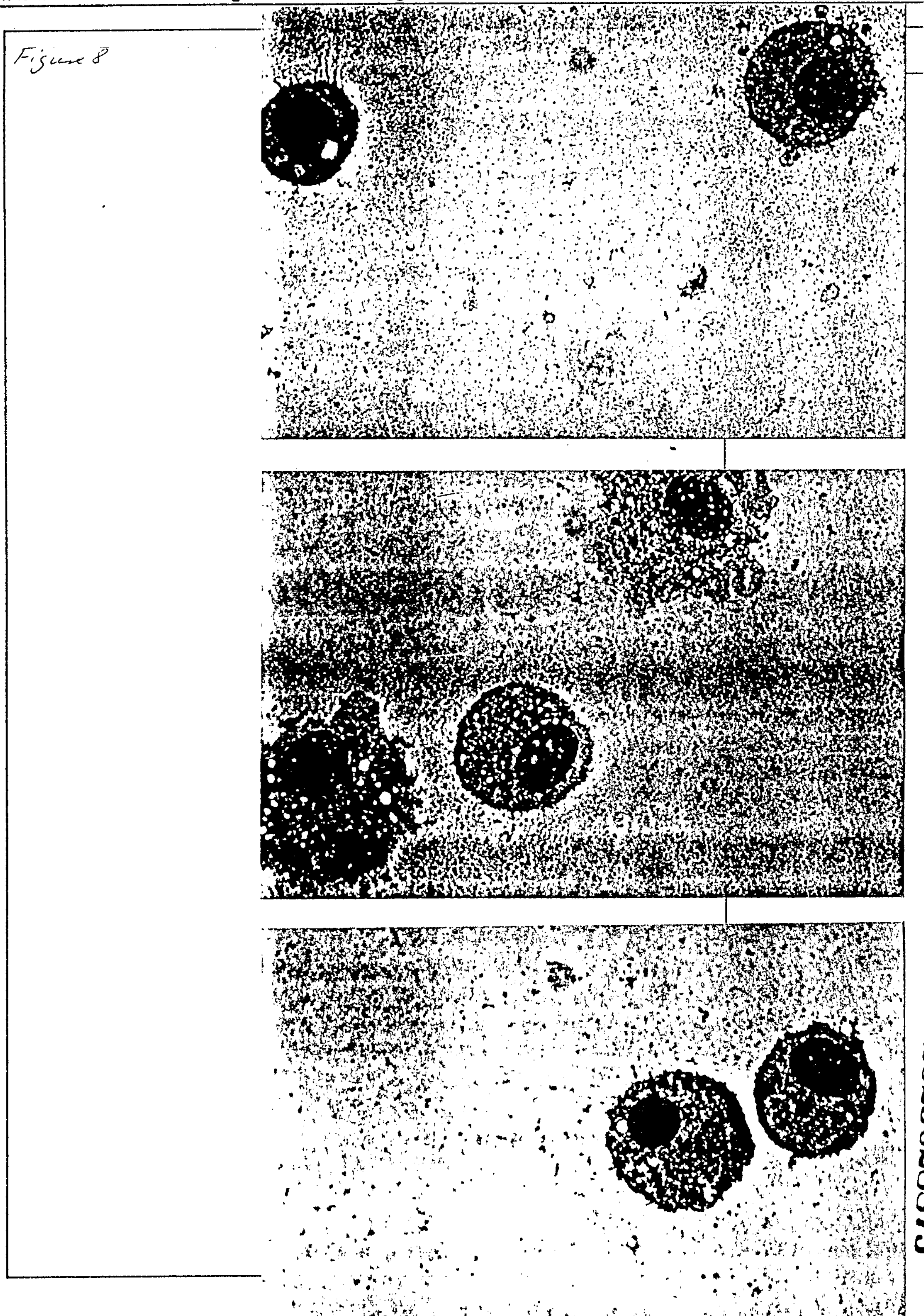
Figure 7

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Figure 8



2029028875

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BC FIGURE 9

SDS-PAGE PROTEIN PATTERNS OF VARIOUS POOLS OF FLC, SILVER STAIN

Remarks:

| SLOT | GROUP | POOL | PROTEIN (ug) |
|---|---|---|---|
| 1 | standards | – | – |
| 2 | 1.2.2 | 4 | 0.91 |
| 3 | standards | – | – |
| 4 | 1.3.2 | 7 | 1.14 |
| 5 | 0.2.3 | 4 | 1.38 |
| 6 | 0.3.3 | 5 | 1.48 |
| 7 | 0.3.3 | 6 | 0.94 |
| 8 | 0.3.3 | 7 | 1.07 |
| 9 | standards | – | – |

SUBREPORT P 0500/3057 GD151 (R) B18 WS M

BC FIGURE 10

SDS-PAGE PROTEIN PATTERNS OF VARIOUS POOLS OF FLC, COOMASSIE BLUE STAIN

Remarks:

| SLOT | GROUP | POOL | PROTEIN (ug) |
|---|---|---|---|
| 1 | - | - | - |
| 2 | 1.1.2 | 2 | 4.86 |
| 3 | 1.2.2 | 4 | 4.24 |
| 4 | 1.3.2 | 7 | 5.31 |
| 5 | 0.2.3 | 4 | 3.21 |
| 6 | 0.3.3 | 7 | 2.49 |
| 7 | 1.2.3 | 4 | 5.26 |
| 8 | 1.3.3 | 7 | 3.51 |
| 9 | 2.2.3 | 4 | 5.09 |
| 10 | 2.3.3 | 7 | 3.21 |
| 11 | standards | - | - |

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SUBREPORT P 0500/3057 GD151 (R) B19 WS M HO3448

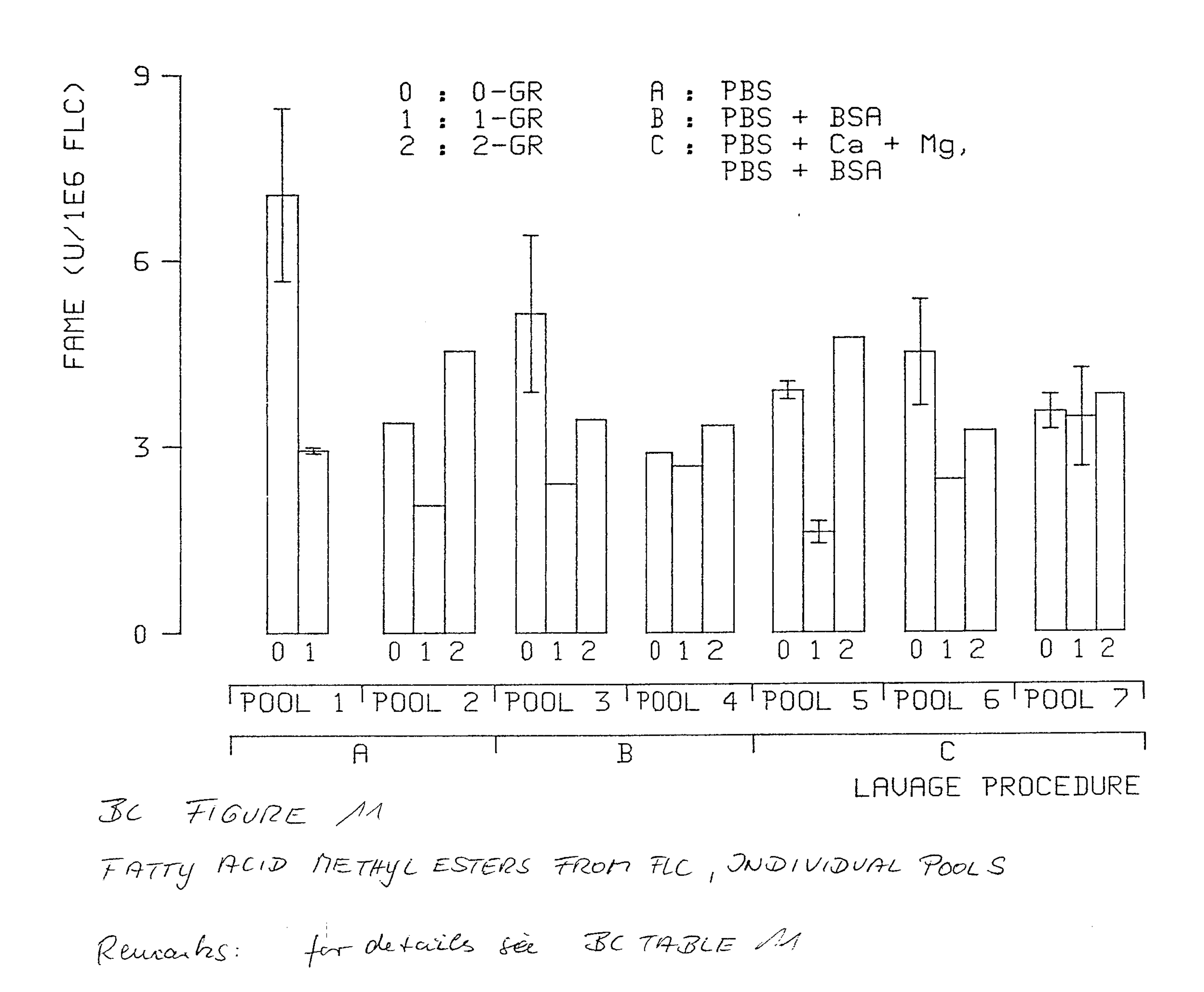
BC FIGURE 11

FATTY ACID METHYL ESTERS FROM FLC, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 11

2029028878

A 0500/3057, H03448, TH, U137 F155 U93




BC FIGURE 11

FATTY ACID METHYL ESTERS FROM FLC, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 11

2029028879

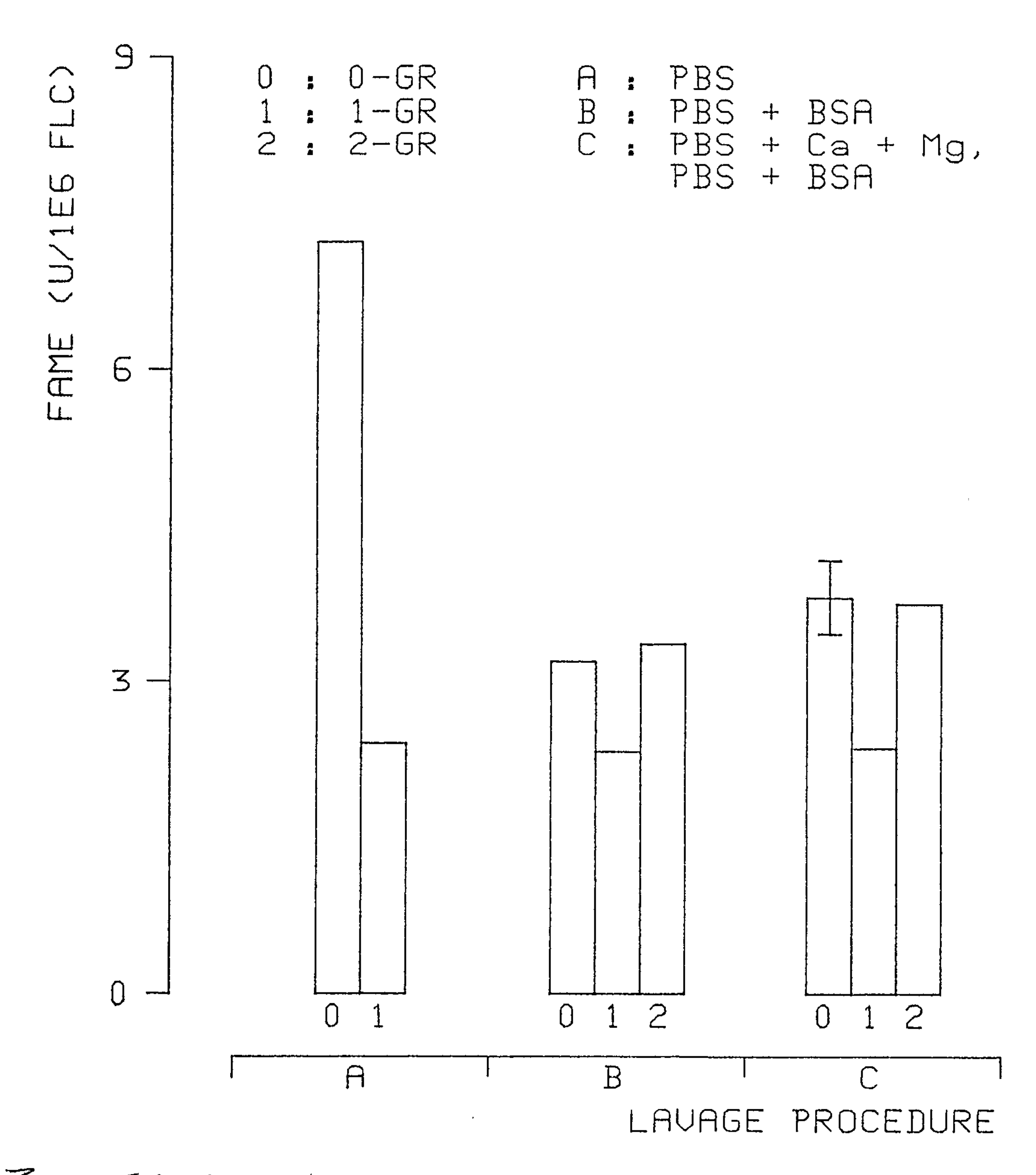
SUBREPORT P 0500/3057 GD151 (R) B19 WS M HO3449

BC FIGURE 12

FATTY ACID METHYL ESTERS FROM FLC, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 11

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BC FIGURE 1.2

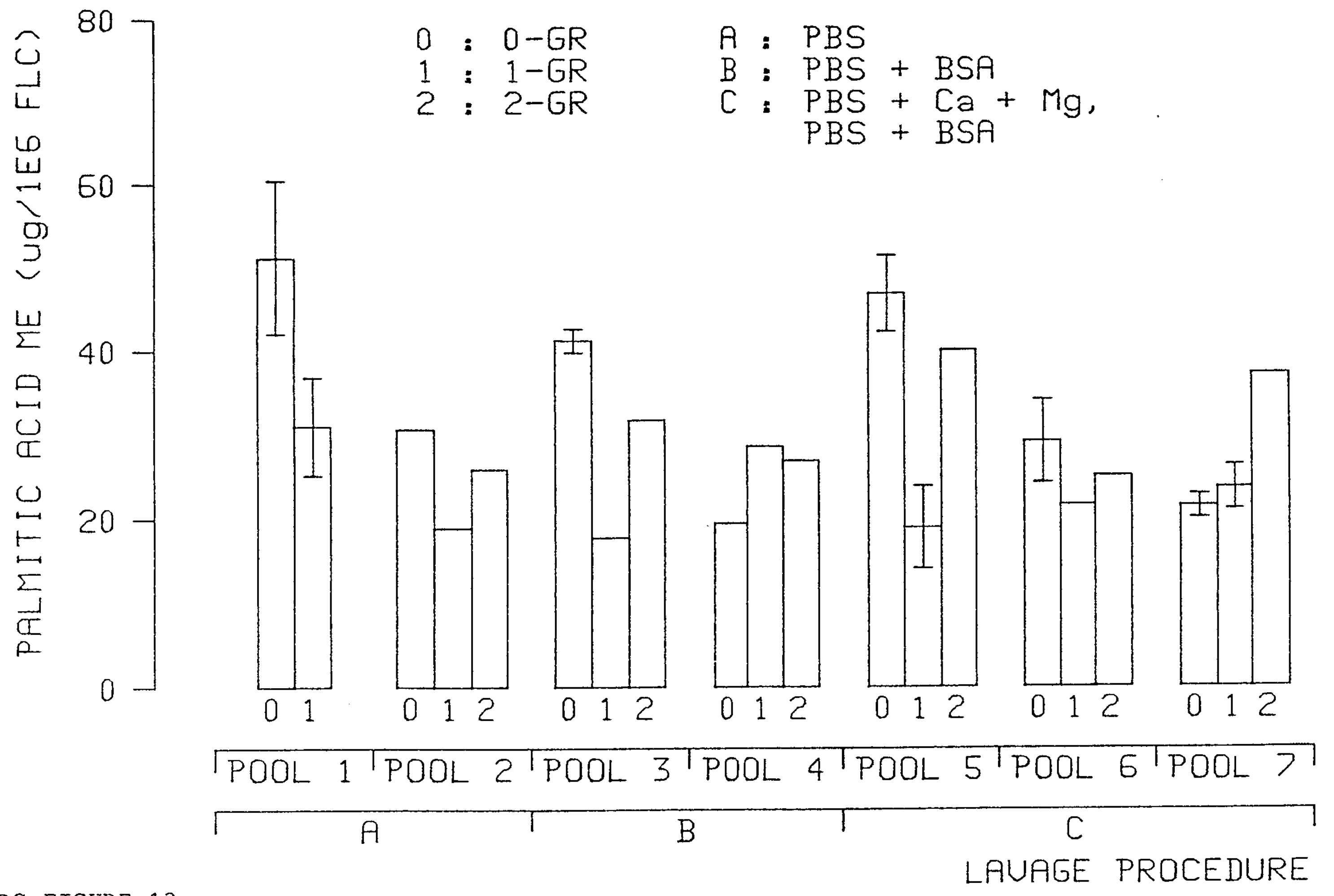
FATTY ACID METHYL ESTERS FROM FLC, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 1.1

A 0500/3057, H03449, TH, V138 F155 V94

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A 0500/3057, H03452, TH, V97 F155 V93
SUBREPORT P 0500/3057 GD151 (R) B19 WS M H03452



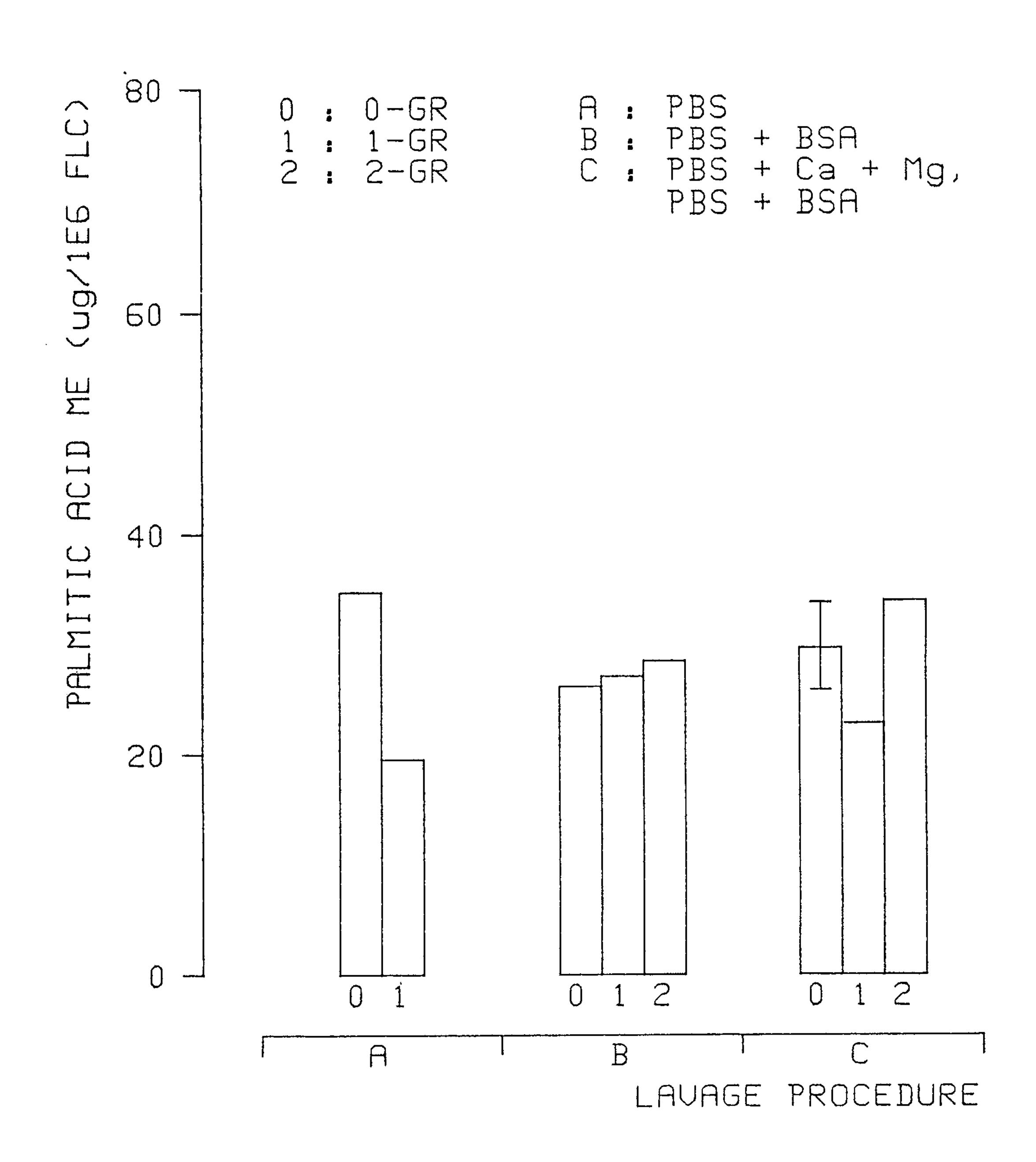



BC FIGURE 13

PALMITIC ACID METHYL ESTER (16 : 0) FROM FLC, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 13

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BC FIGURE 14

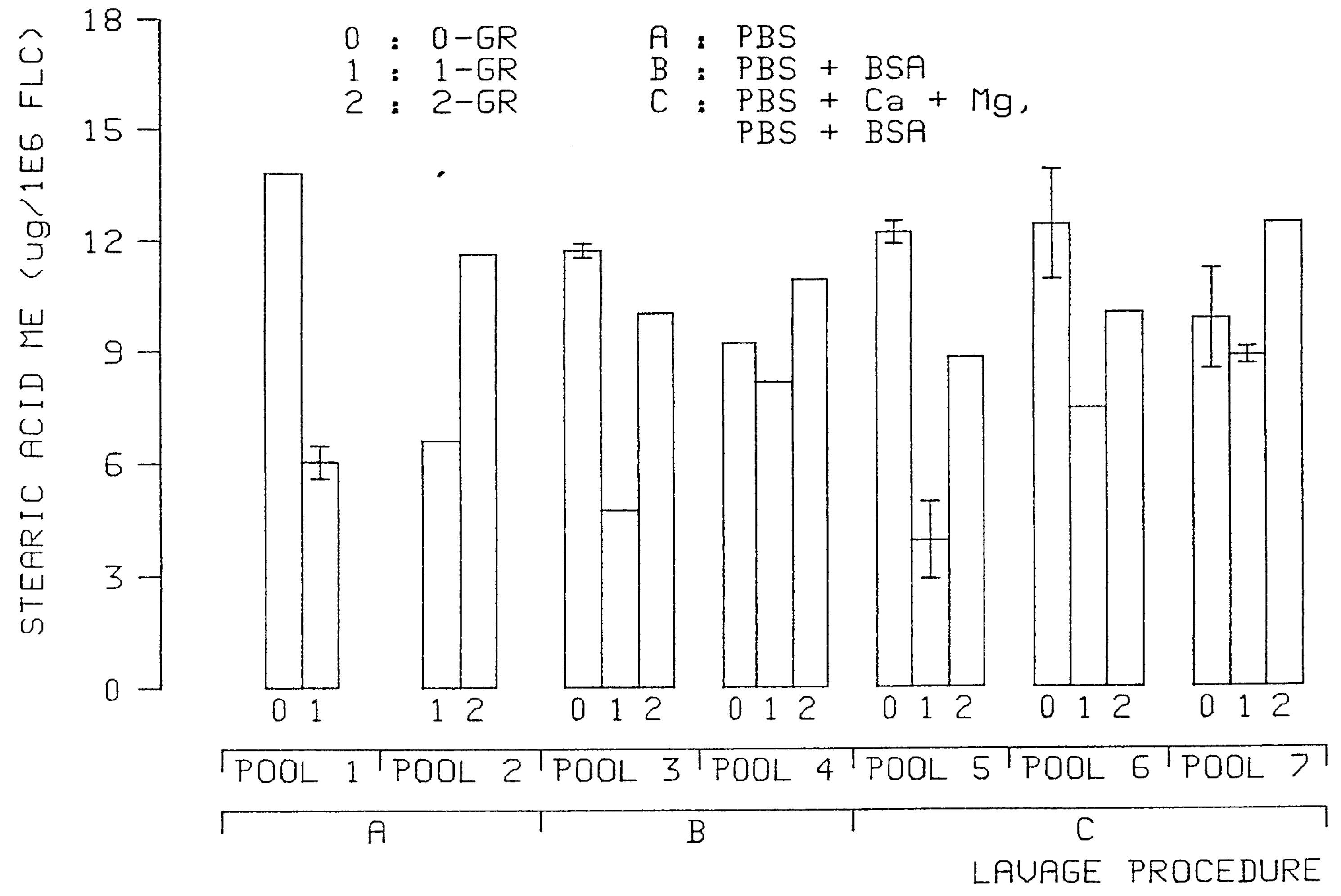
PALMITIC ACID METHYL ESTER (16 : 0) FROM FLC, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 13

A 0500/3057, HO3453, TH, V98 F155 V90

2029028883

A 0500/3057, HO3450, TH, V95 F155 V93,
SUBREPORT P 0500/3057 GD151 (R) B19 WS M HO3450



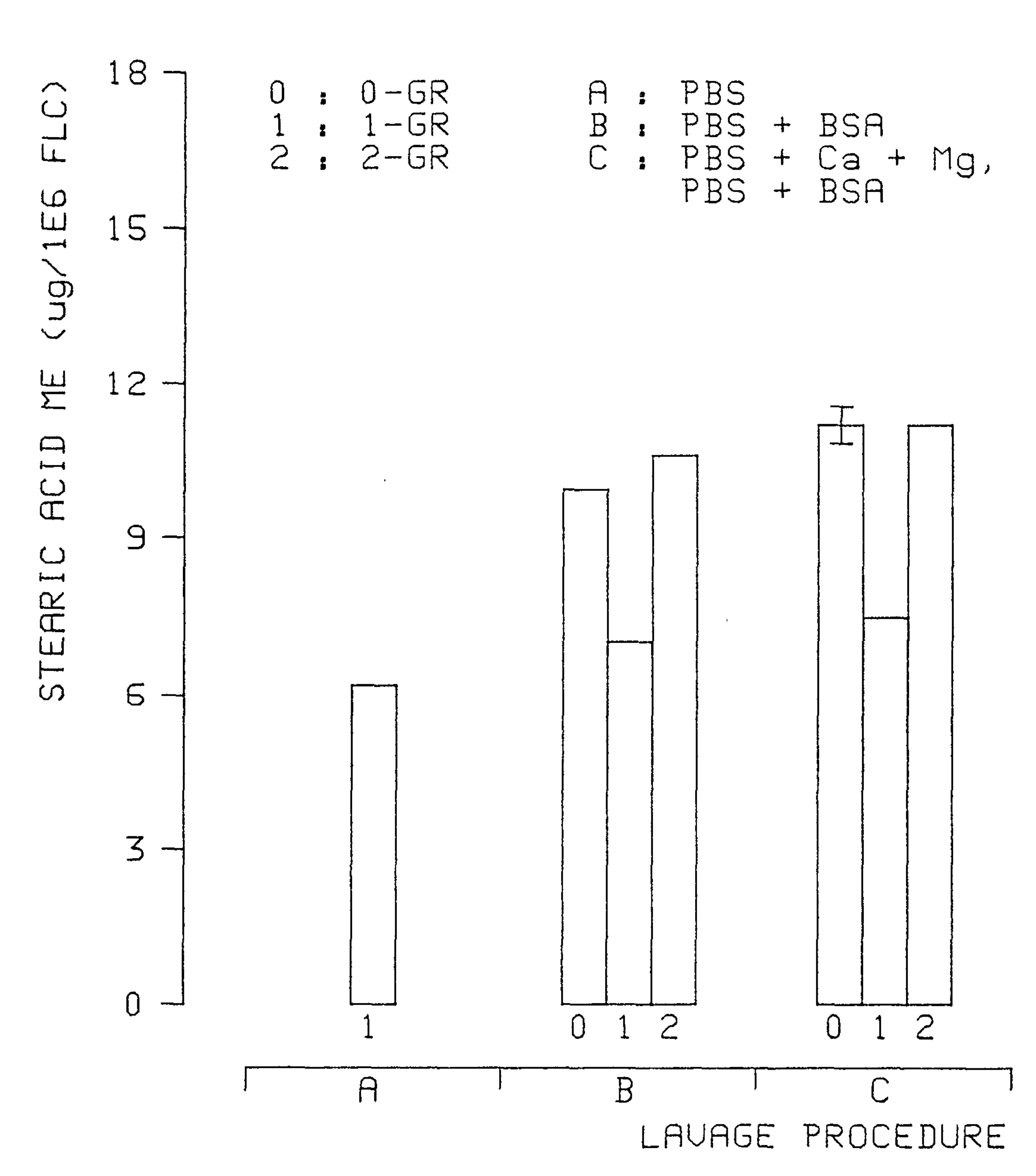



BC FIGURE 15

STEARIC ACID METHYL ESTER (18 : 0) FROM FLC, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 15

2029028884




BC FIGURE 16

STEARIC ACID METHYL ESTER (18 : 0) FROM FLC, WEIGHTED MEANS OF POOLS

Remarks: for details see BĊ TABLE 15

A 0500/3057, HO3451, TH, V123 F155 V96

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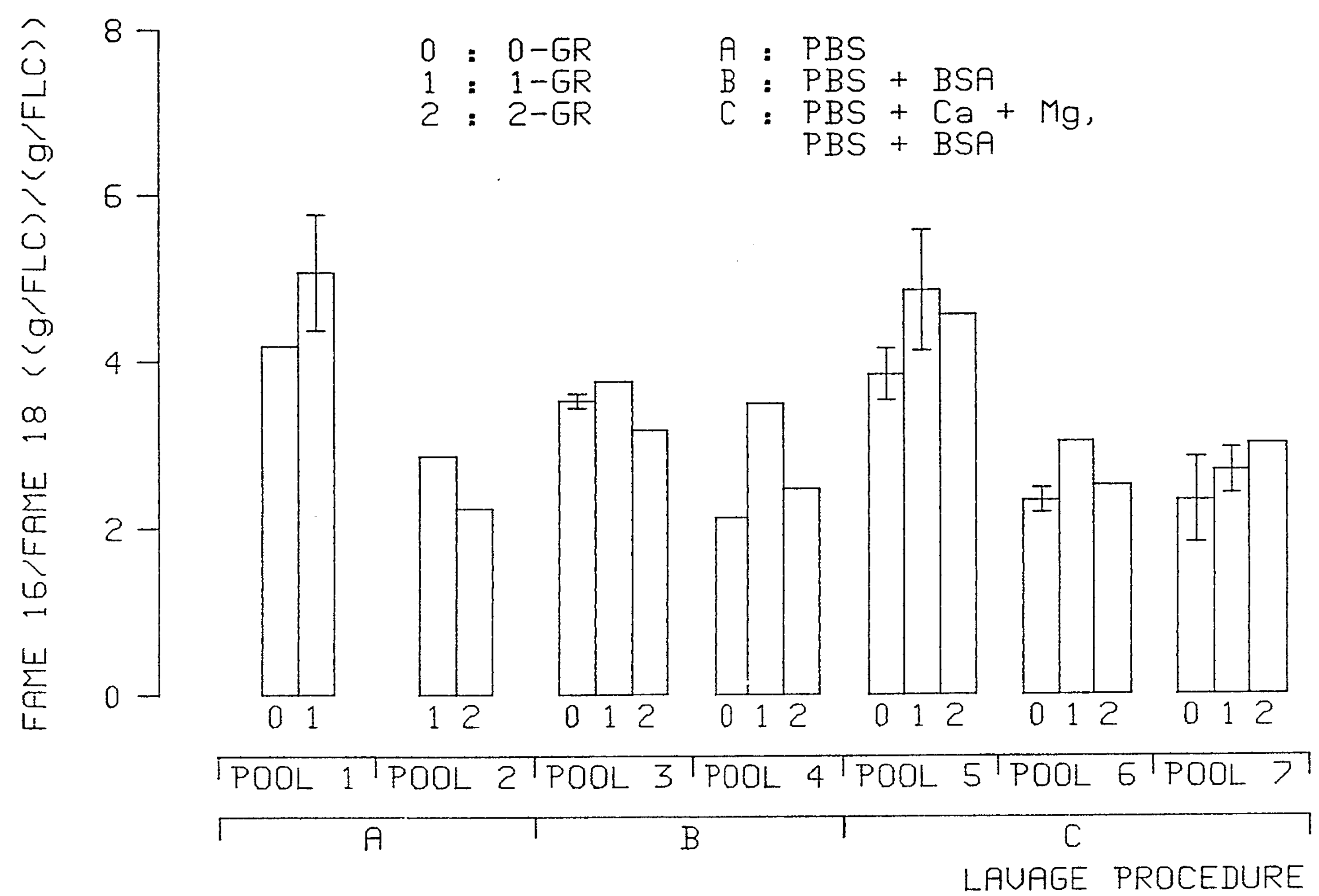
SUBREPORT P 0500/3057   GD151 (R) B20   WS   M   H03454

BC FIGURE 17

RATIO OF PALMITIC ACID METHYL ESTER (16 : 0) VERSUS STEARIC ACID METHYL ESTER (18 : 0), INDIVIDUAL POOLS

Remarks: for details see BC TABLE 16

A 0500/3057, H03454, TH, V140 F155 V120




BC FIGURE 17

RATIO OF PALMITIC ACID METHYLESTER (16:0) VERSUS STEARIC ACID METHYLESTER (18:0), INDIVIDUAL POOLS

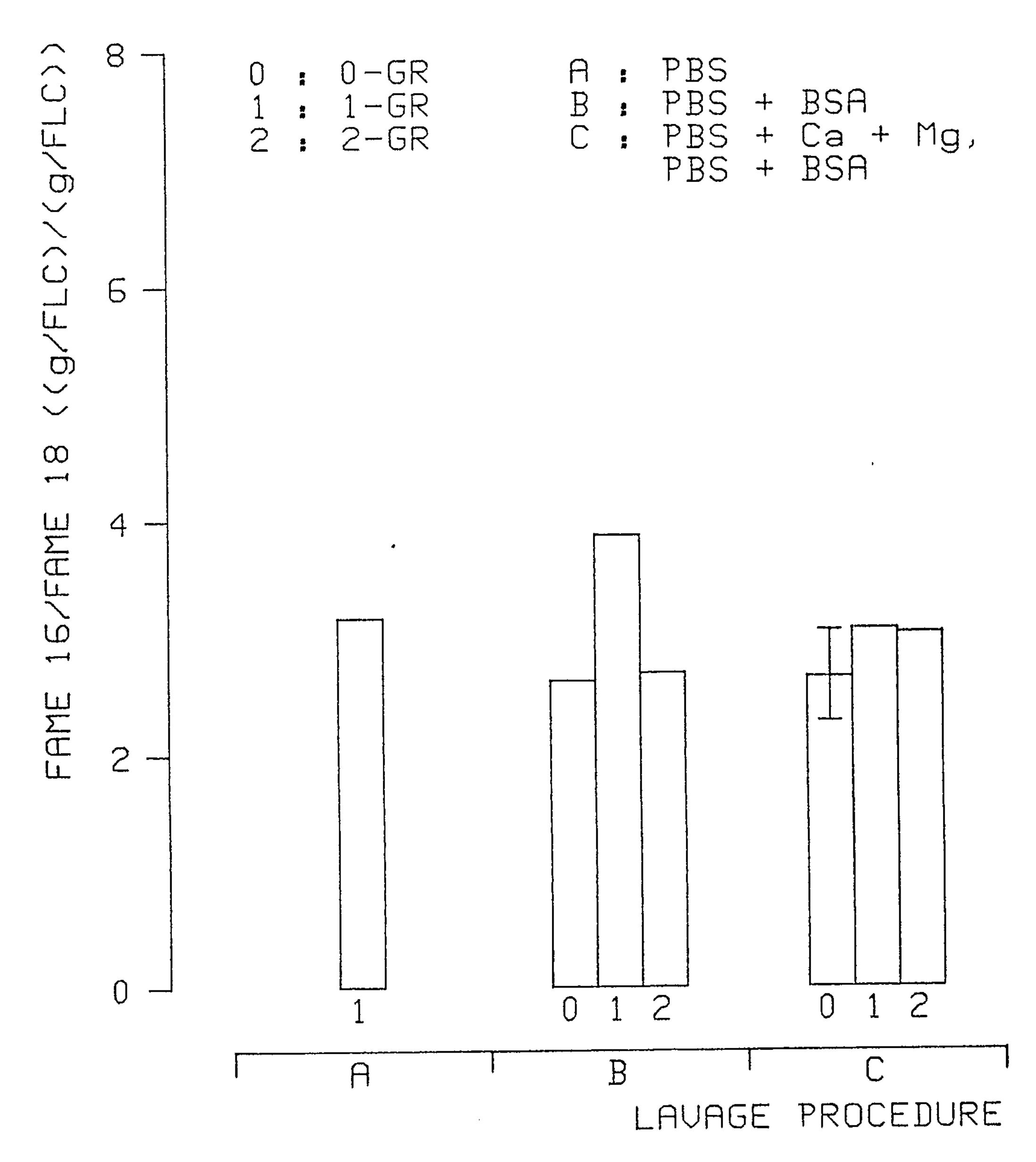
Remarks: for details see BC TABLE 16

2029028887

BC FIGURE 18

RATIO OF PALMITIC ACID METHYL ESTER (16 : 0) VERSUS STEARIC ACID METHYL ESTER (18 : 0), WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 16




BC FIGURE 18

RATIO OF PALMITIC ACID METHYLESTER (16:0) VERSUS STEARIC ACID METHYL ESTER (18:0), WEIGHTED MEANS OF POOLS

Remarks : for details see BC TABLE 16

A 0500/3057, H03455, TH, U141 F155 U121

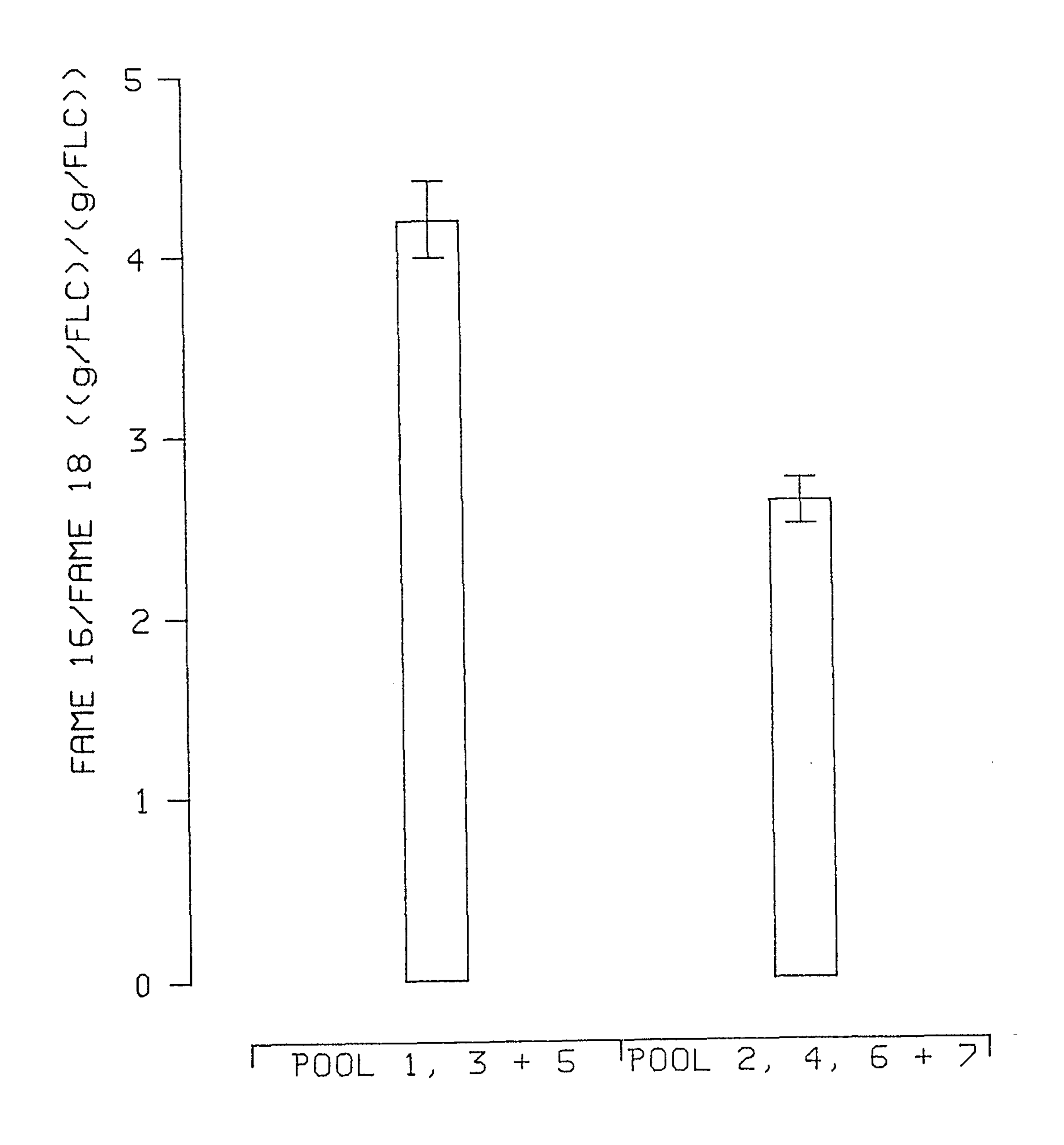
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BC FIGURE 19

MEAN RATIO OF PALMITIC ACID METHYL ESTER (16 : 0) VERSUS STEARIC ACID METHYL ESTER (18 : 0), EARLY AND LATE POOLS

Remarks: for details see BC TABLE 17

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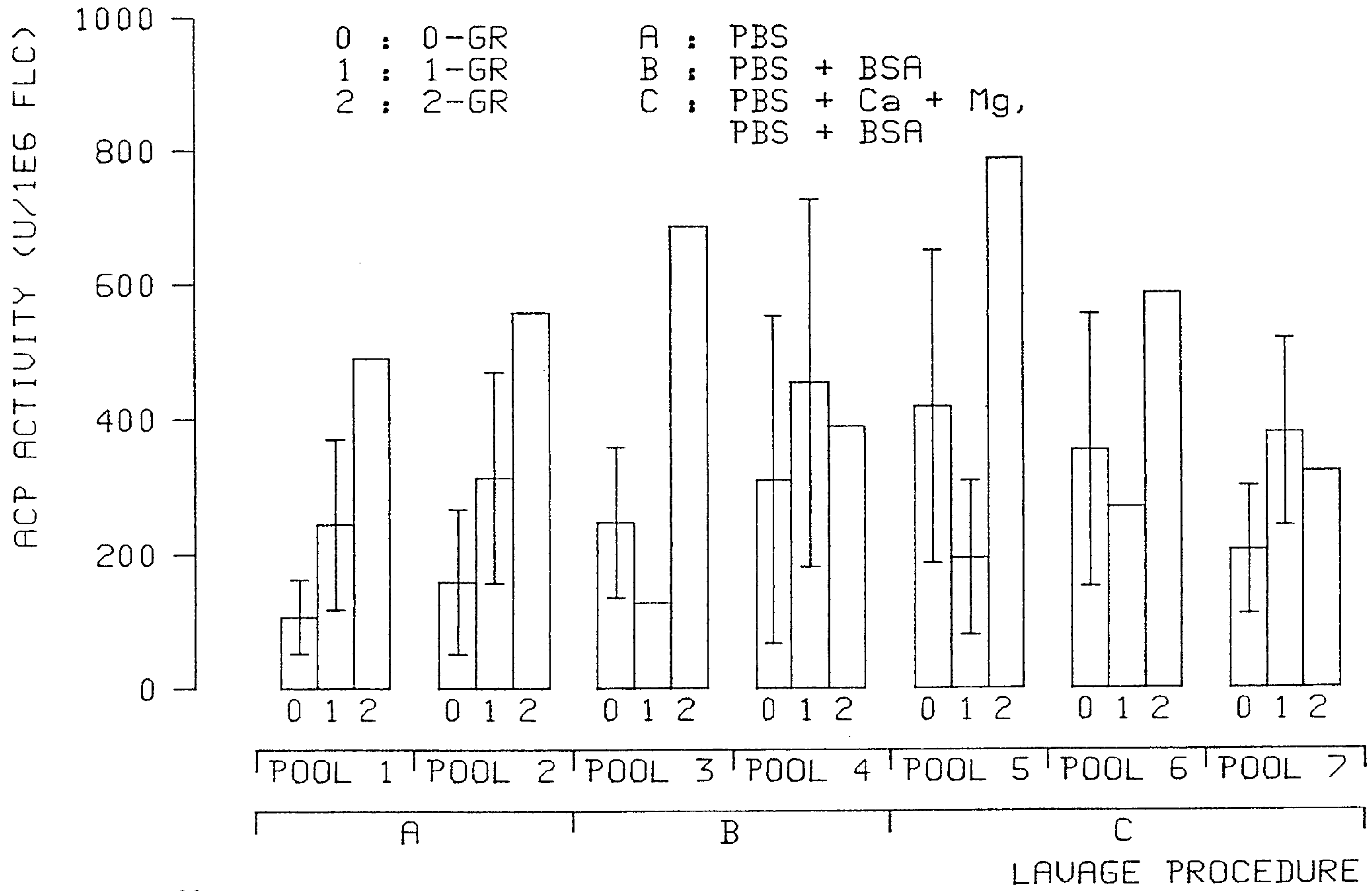

BC FIGURE 19

MEAN RATIO OF PALMITIC ACID METHYL ESTER (16:0) VERSUS STEARIC ACID METHYLESTER (18:0), EARLY AND LATE POOLS

Remarks: for details see BC TABLE 17

A 0500/3057, H03471, TH, V139 F155 V122

A 0500/3057, H03447, TH, V119 F155 V92
SUBREPORT P 0500/3057 GD151 (R) B20 WS M H03447



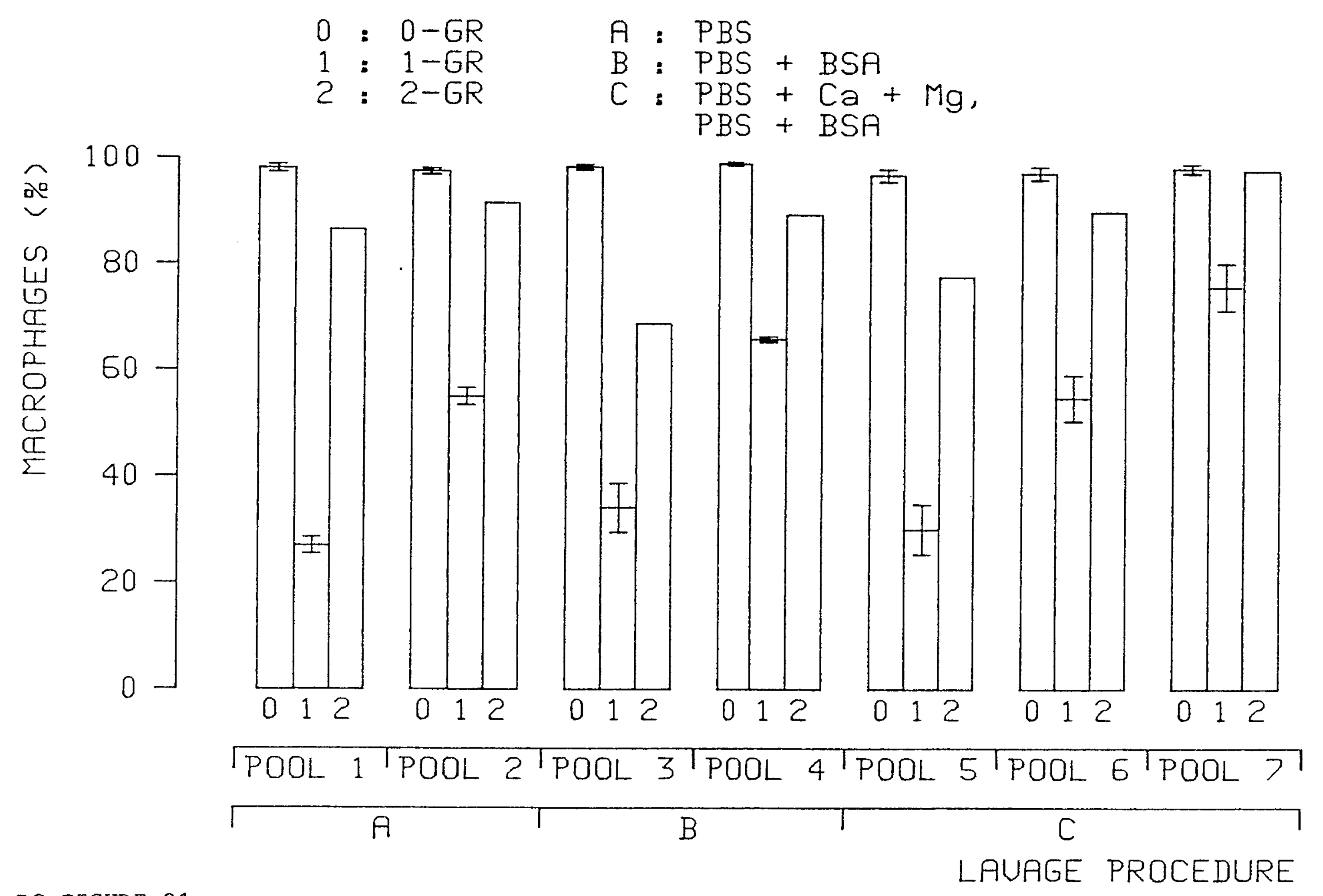



BC FIGURE 20

ACID PHOSPHATASE ACTIVITY, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 21

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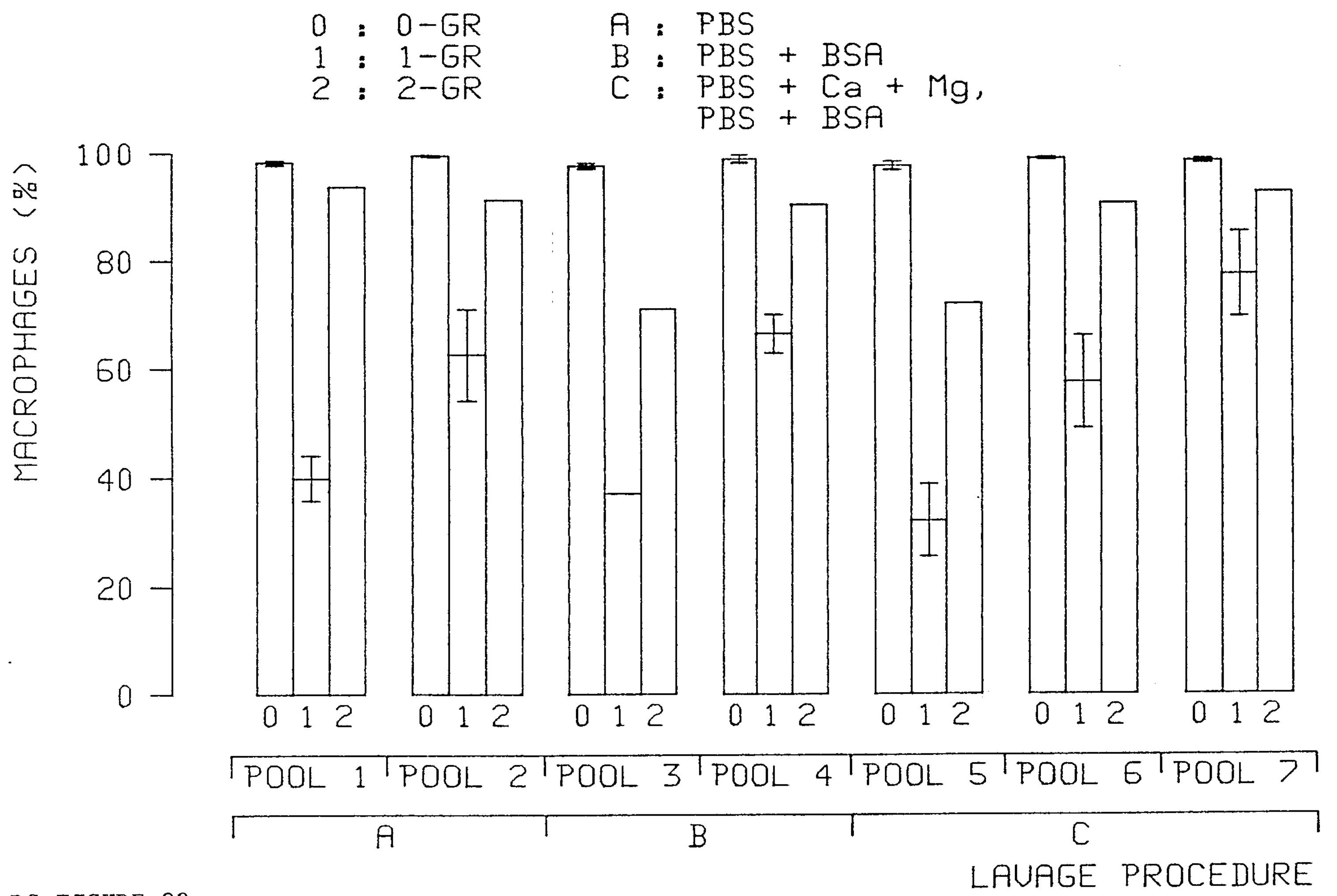



BC FIGURE 21

RELATIVE NUMBER OF MACROPHAGES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 24

A 0500/3057, H03212, TH, V84 F155 V83, R
SUBREPORT P 0500/3057 GD151 (R) B21 WS M H03212

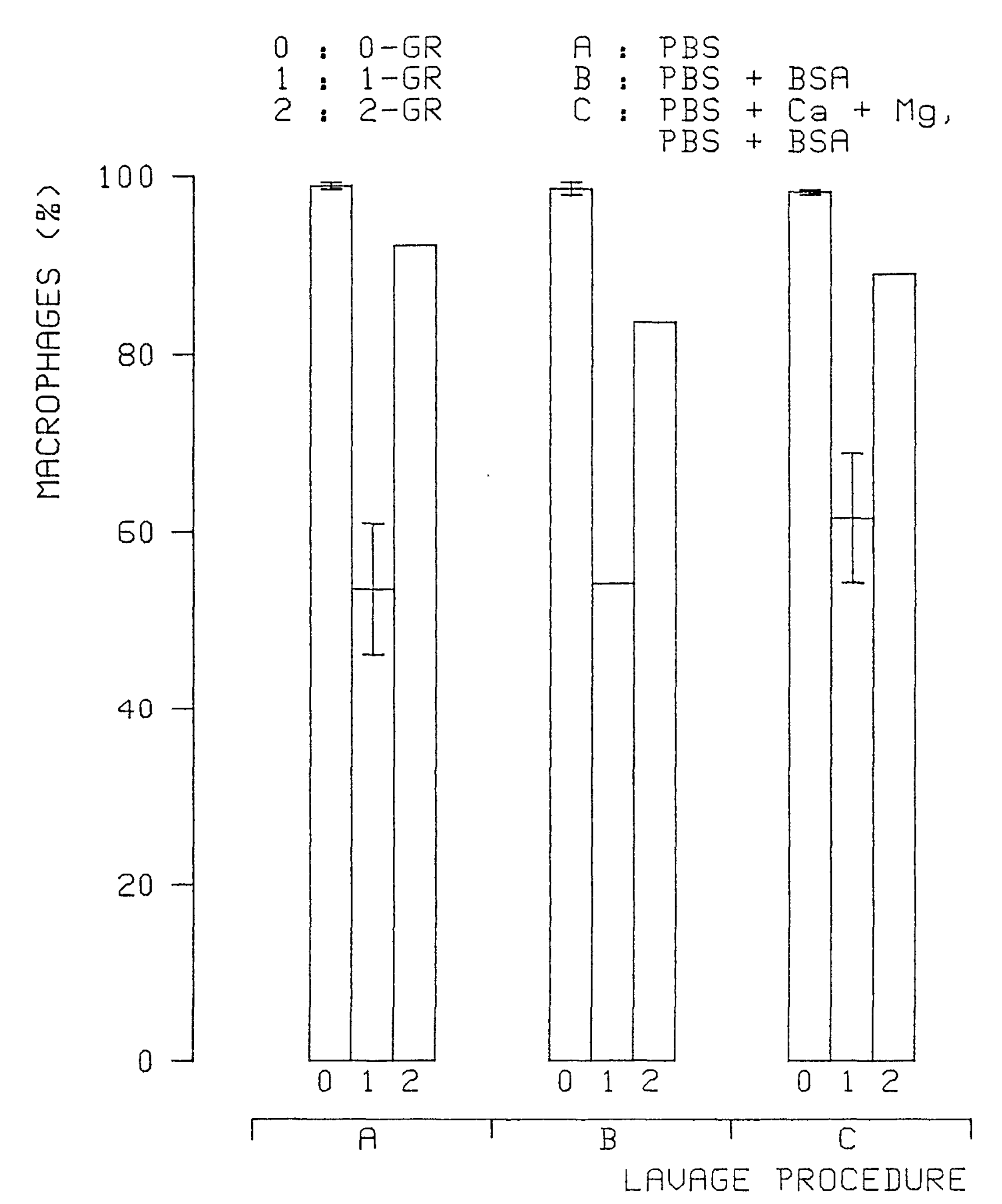





BC FIGURE 22

RELATIVE NUMBER OF MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 26



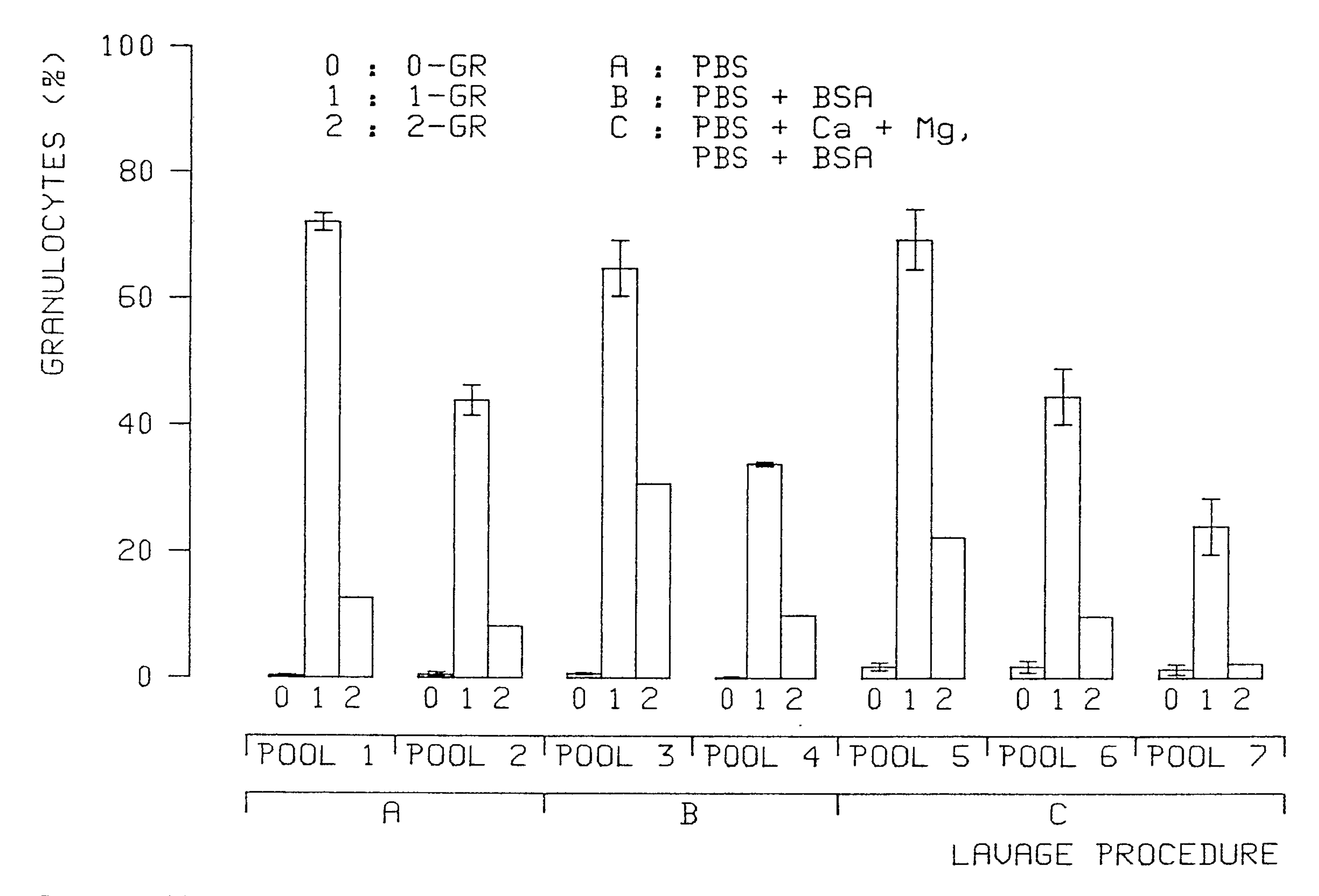

BC FIGURE 23

RELATIVE NUMBER OF MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 26

A 0500/3057, H03215, TH, V85 F155 V82, R

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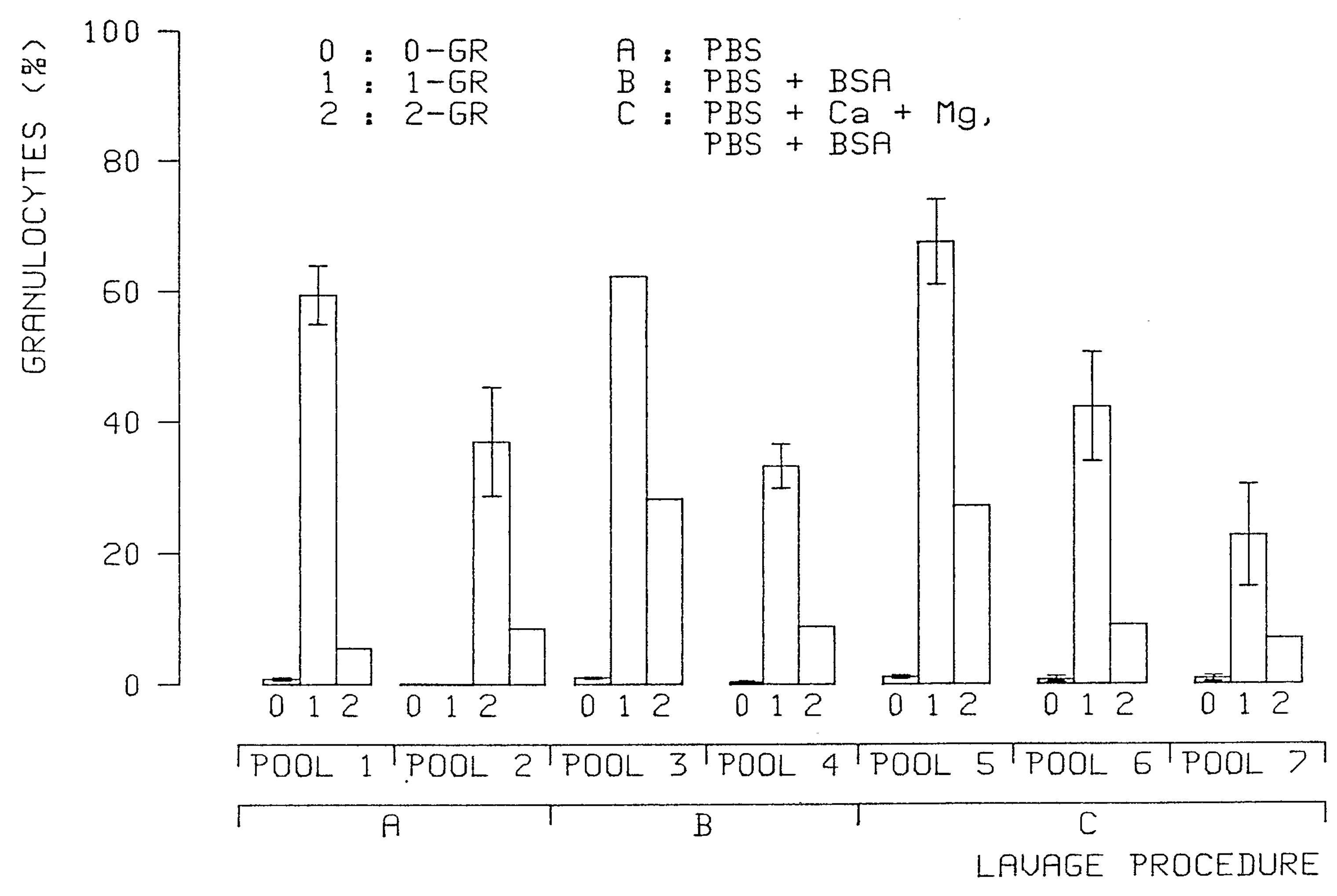



BC FIGURE 24

RELATIVE NUMBER OF GRANULOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 28

A 0500/3057, H03216, TH, V75 F155 V74, R
SUBREPORT P 0500/3057 GD151 (R) B21 WS M H03216

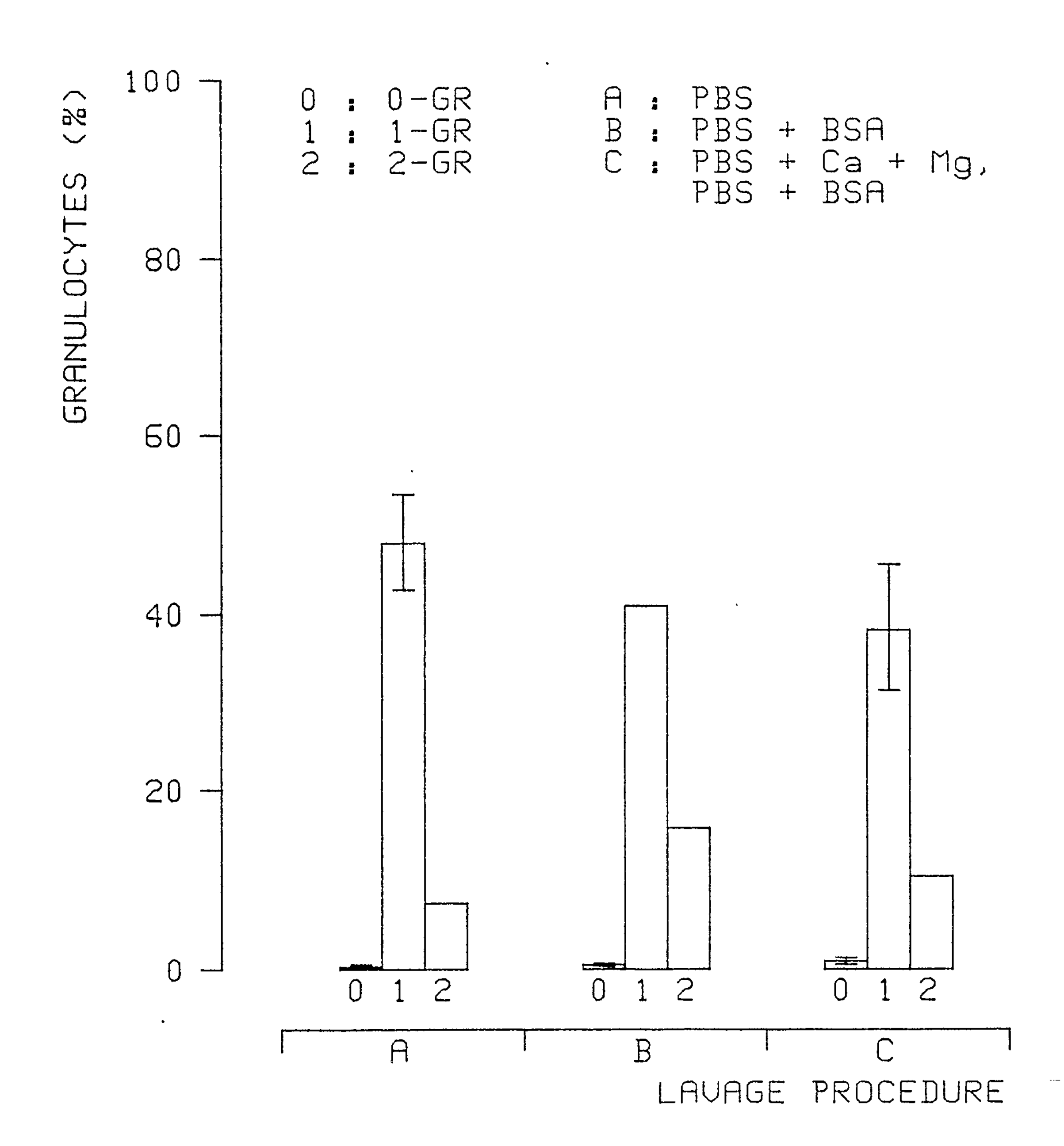





BC FIGURE 25

RELATIVE NUMBER OF GRANULOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 30




BC FIGURE 26

RELATIVE NUMBER OF GRANULOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, WEIGHTED MEANS OF POOLS

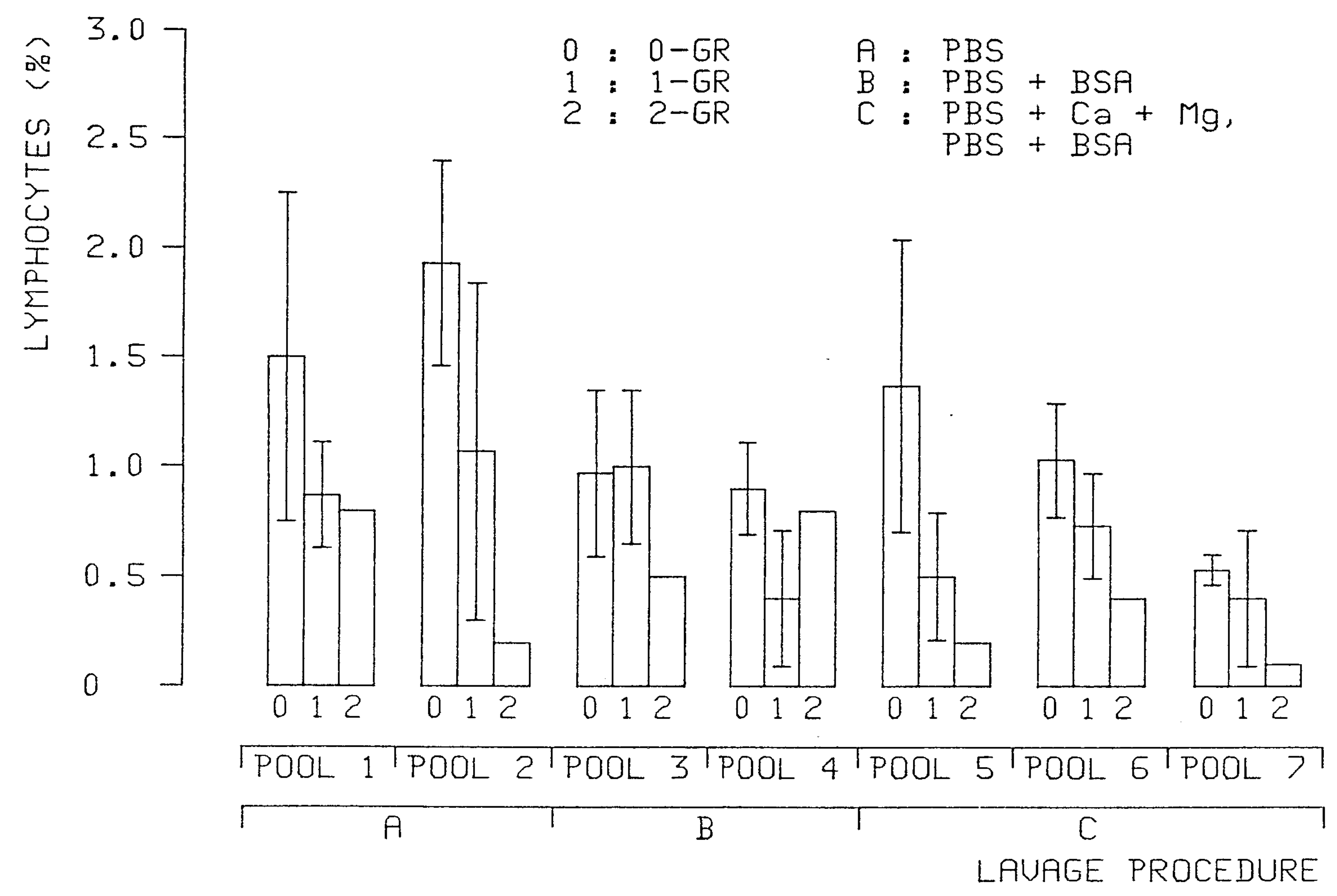
Remarks: for details see BC TABLE 30

A 0500/3057, H03219, TH, V76 F155 V72, R

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A 0500/3057, H03207, TH, V77 F155 V75, L

SUBREPORT P 0500/3057 GD151 (R) B22 WS M H03207



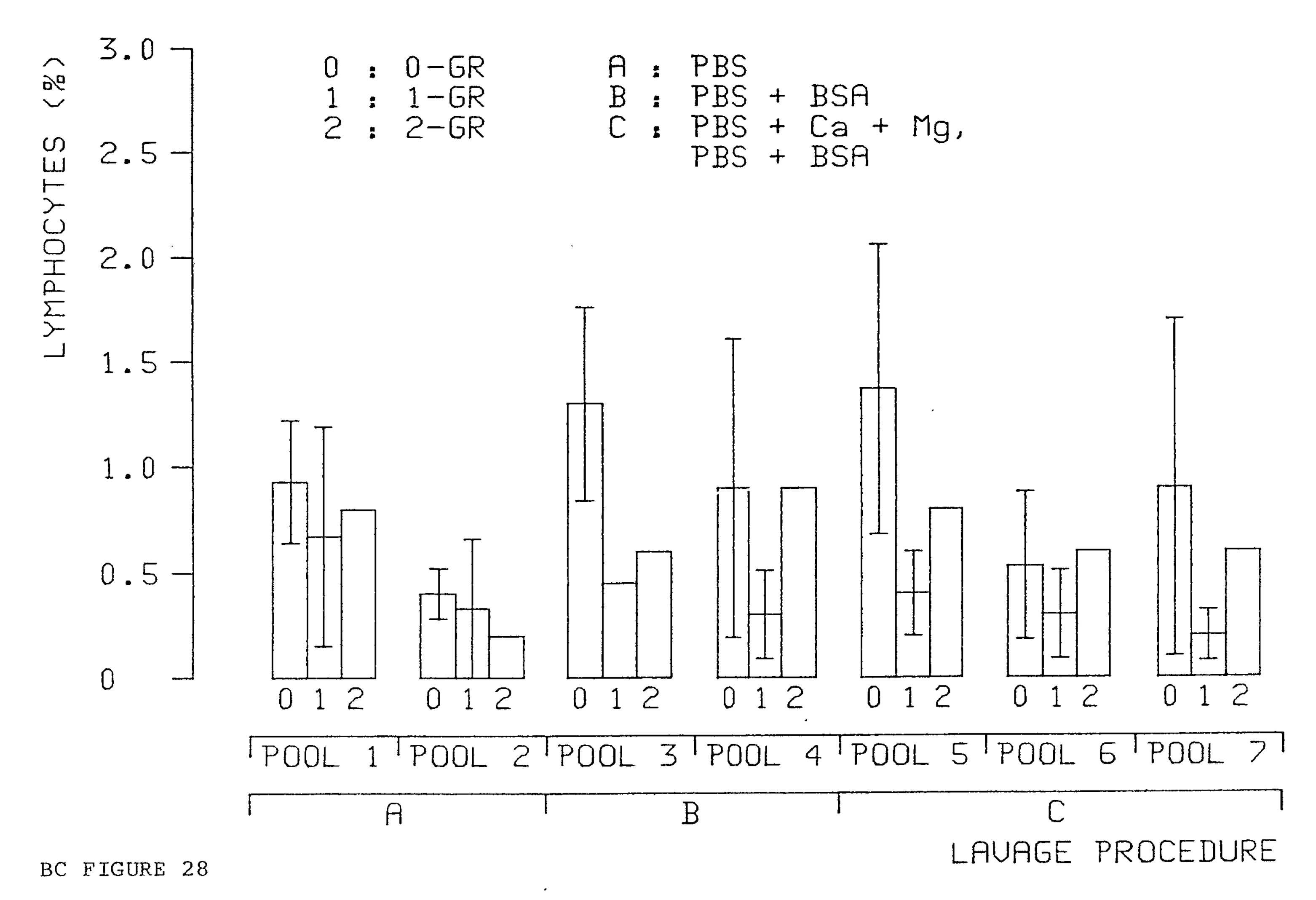

BC FIGURE 27

RELATIVE NUMBER OF LYMPHOCYTES, LAVAGE MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 32

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BC FIGURE 28

RELATIVE NUMBER OF LYMPHOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 34

LYMPHOCYTES (%)

3.0
2.5
2.0
1.5
1.0
0.5
0

0 : 0-GR
1 : 1-GR
2 : 2-GR

A : PBS
B : PBS + BSA
C : PBS + Ca + Mg,
PBS + BSA

0 1 2 | 0 1 2 | 0 1 2

A B C

LAVAGE PROCEDURE




BC FIGURE 29

RELATIVE NUMBER OF LYMPHOCYTES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 34

2029028901

SUBREPORT P 0500/3057 GD151 (R) B22 WS M H03456

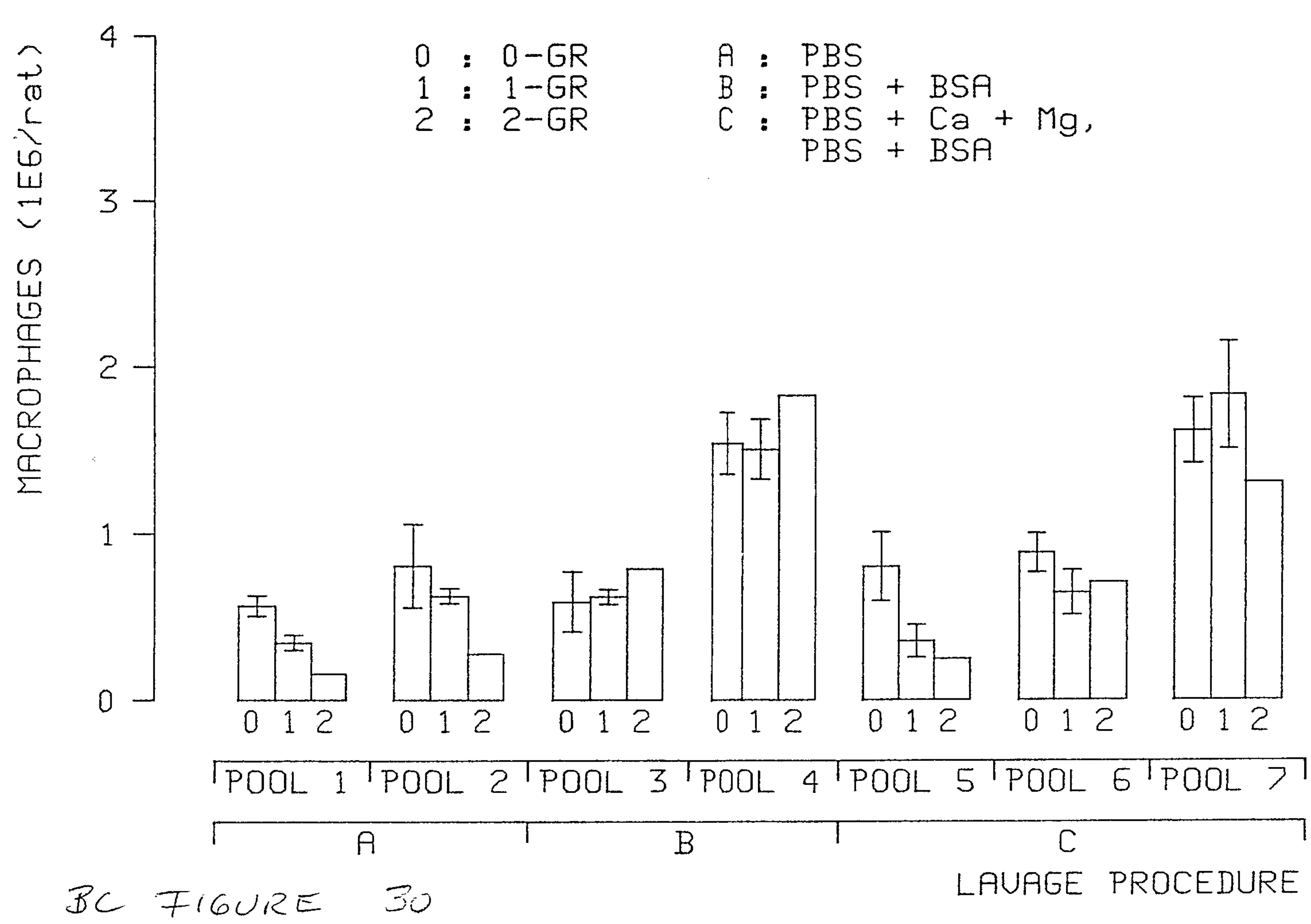
BC FIGURE 30

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 36

2029028902

A 0500/3057, H03456, TH, V136 F155 V101 R




BC FIGURE 30

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMO CYTOMETER, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 36

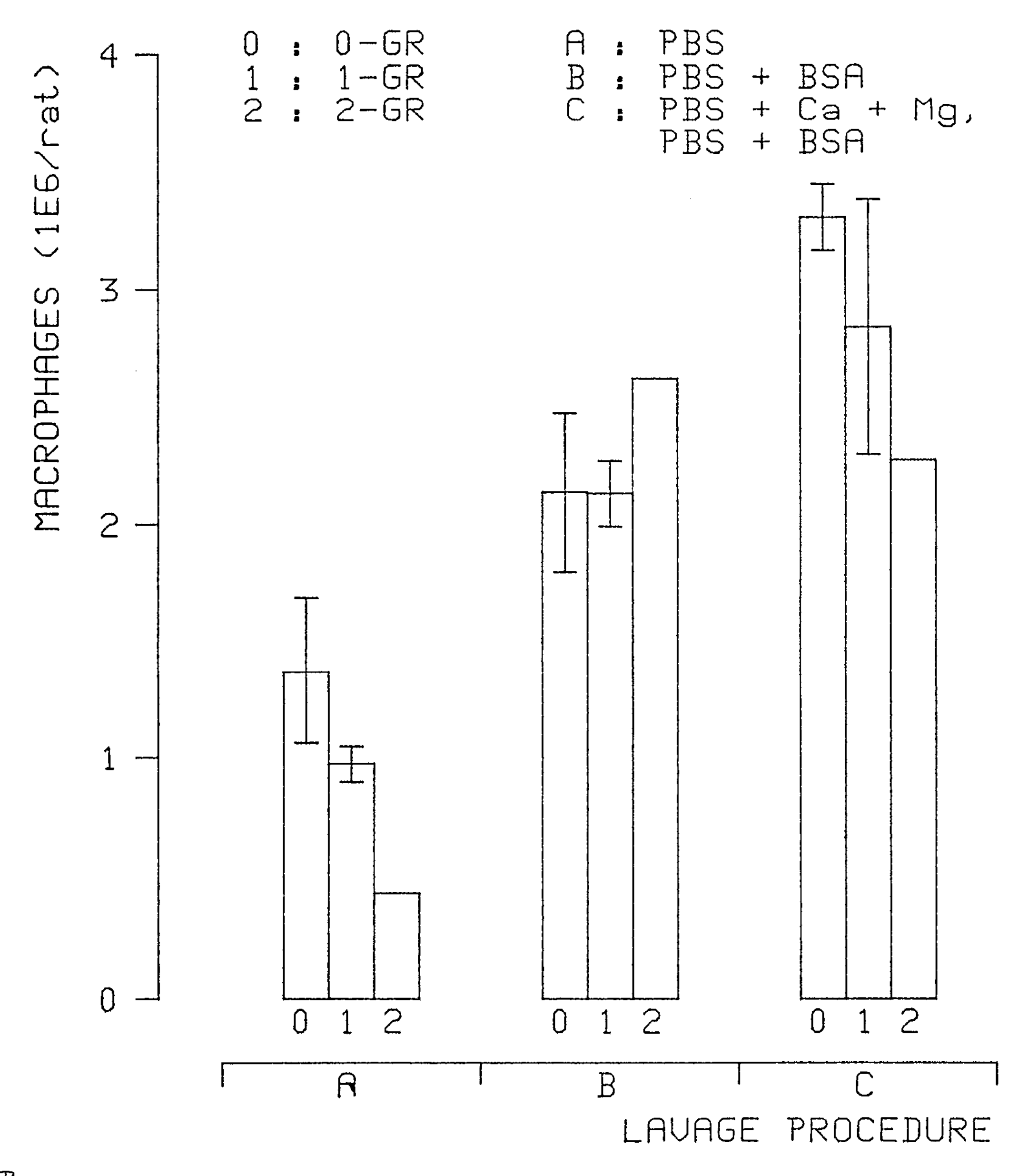
2029028903

SUBREPORT P 0500/3057 GD151 (R) B23 WS M HO3457

BC FIGURE 31

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, SUM OF POOLS
Remarks: for details see BC TABLE 36

2029028904




BC FIGURE 31

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, SUM OF POOLS

Remarks: for details see BC TABLE 36

A 0500/3057, H03457, TH, V130 F155 U102 R

2029028905

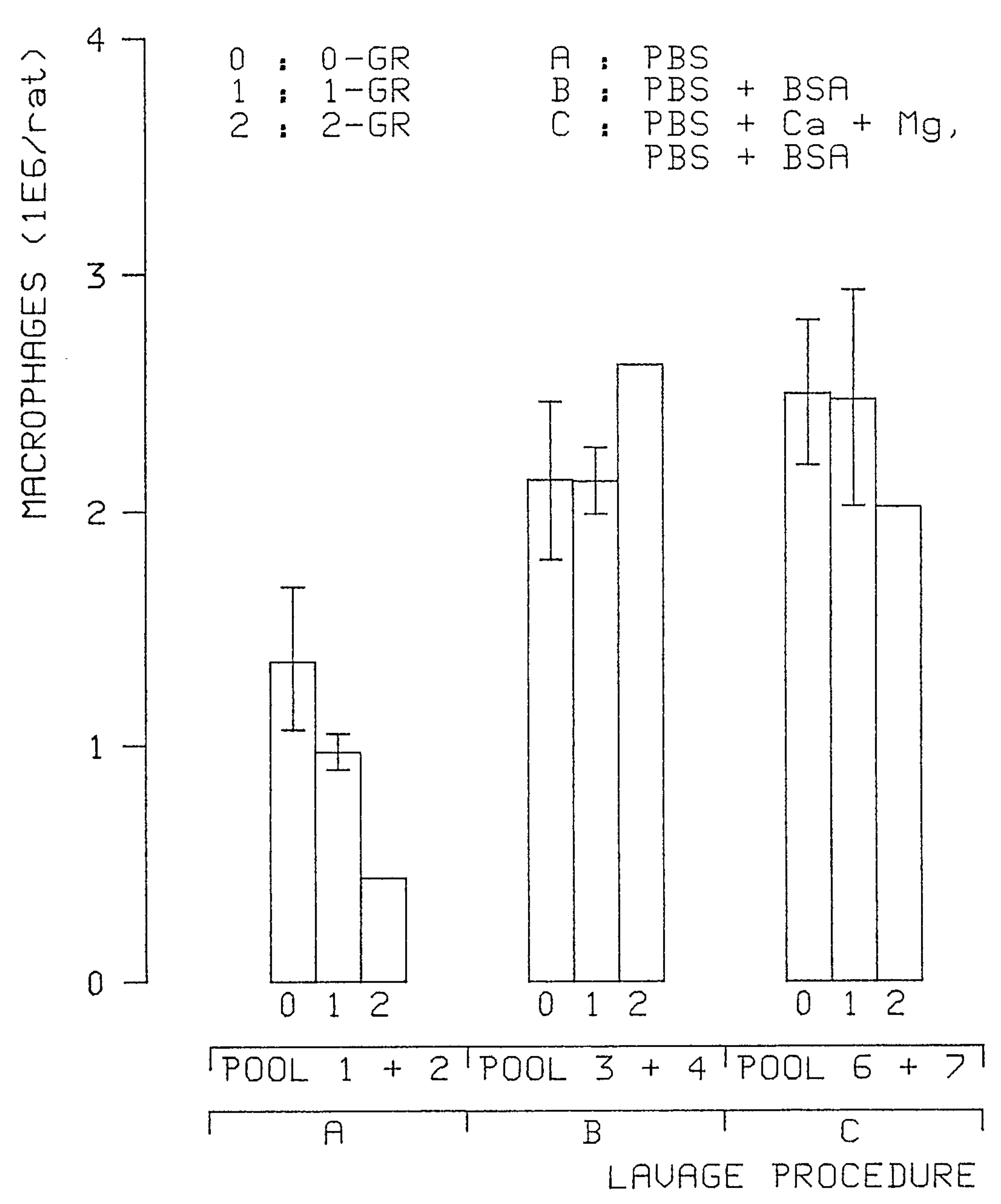
SUBREPORT P 0500/3057 GD151 (R) B23 WS M HO3458

BC FIGURE 31 (continued)

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, SUM OF POOLS

Remarks: for details see BC TABLE 36

2029028906




BC FIGURE 3-1 continued

NUMBER OF MACROPHAGES PER RAT, RESUSPENSION MEDIUM, HEMOCYTOMETER, SUM OF POOLS

Remarks: for details see BC TABLE 3-1

A 0500/3057, H03458, TH, V131 F155 V103 R

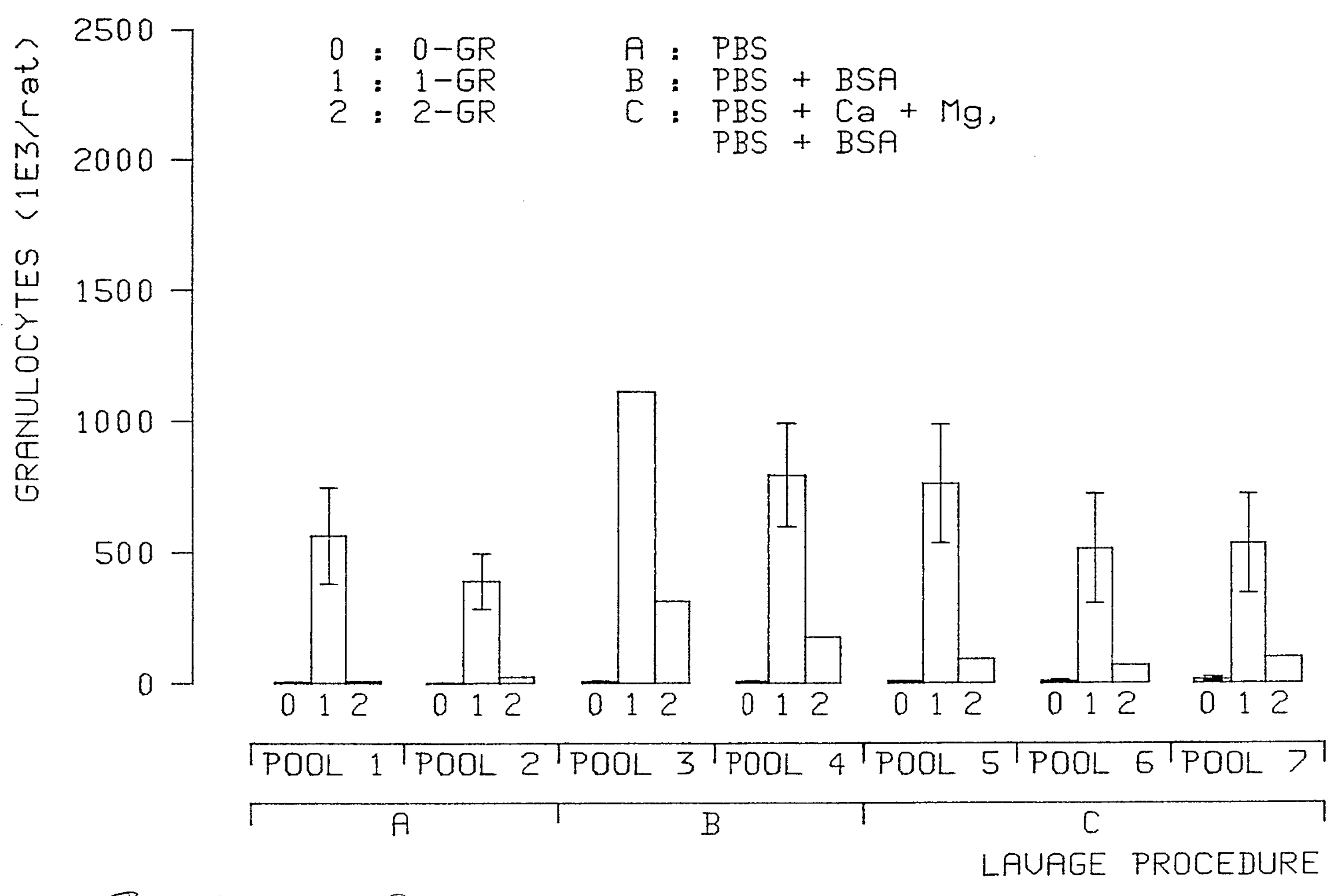
2029028907

BC FIGURE 32

NUMBER OF GRANULOCYTES PER RAT, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 38

A 0500/3057, H03231, TH, V132 F155 V73 R




BC FIGURE 32

NUMBER OF GRANULOCYTES PER RAT, RESUSPENSION MEDIUM, CYTO CENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 32

2029028909

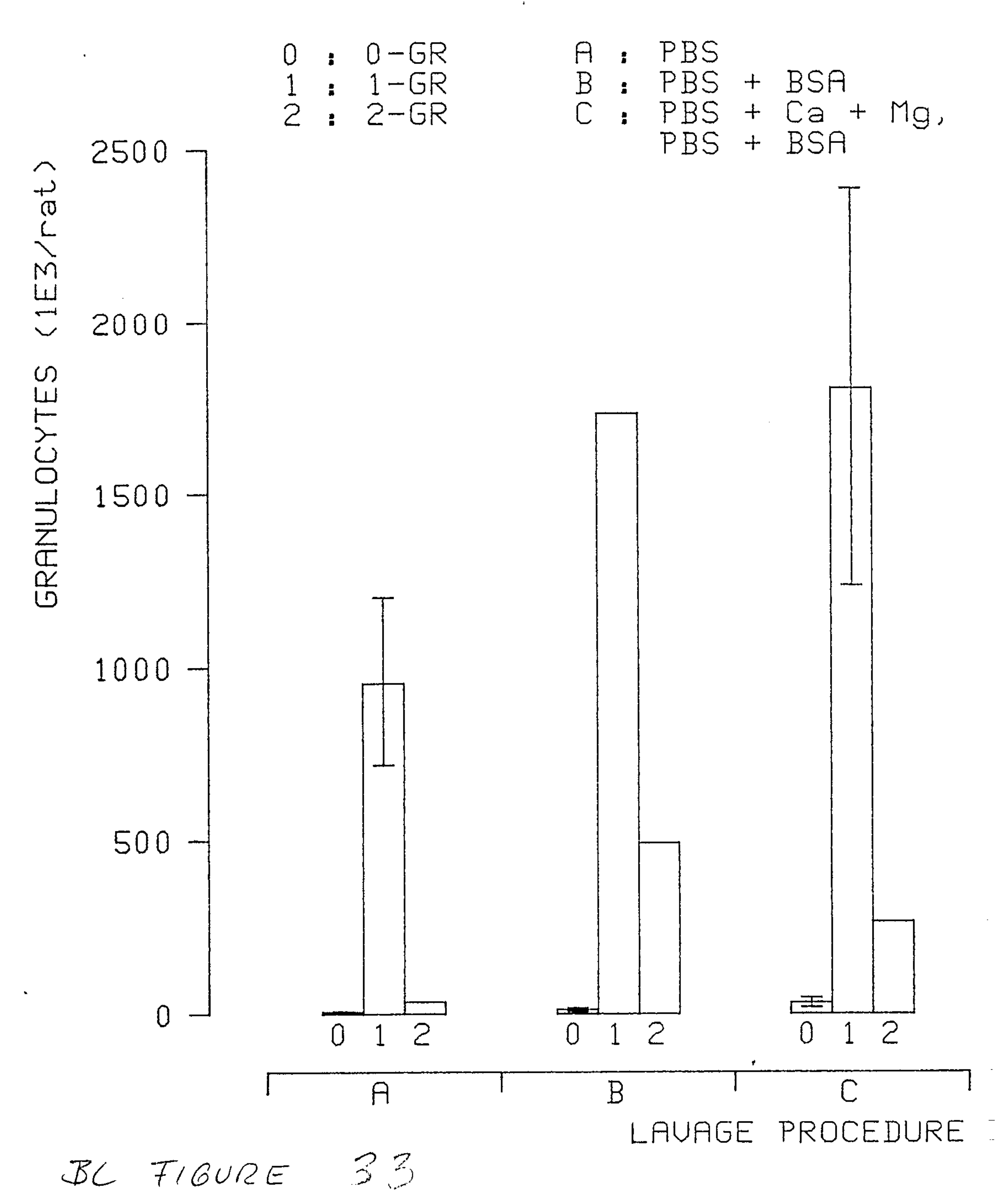
SUBREPORT P 0500/3057 GD151 (R) B23 WS M HO3228

BC FIGURE 33

NUMBER OF GRANULOCYTES PER RAT, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, SUM OF POOLS

Remarks: for details see BC TABLE 38

2029028910




BC FIGURE 33

NUMBER OF GRANULOCYTES PER RAT, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, SUM OF POOLS

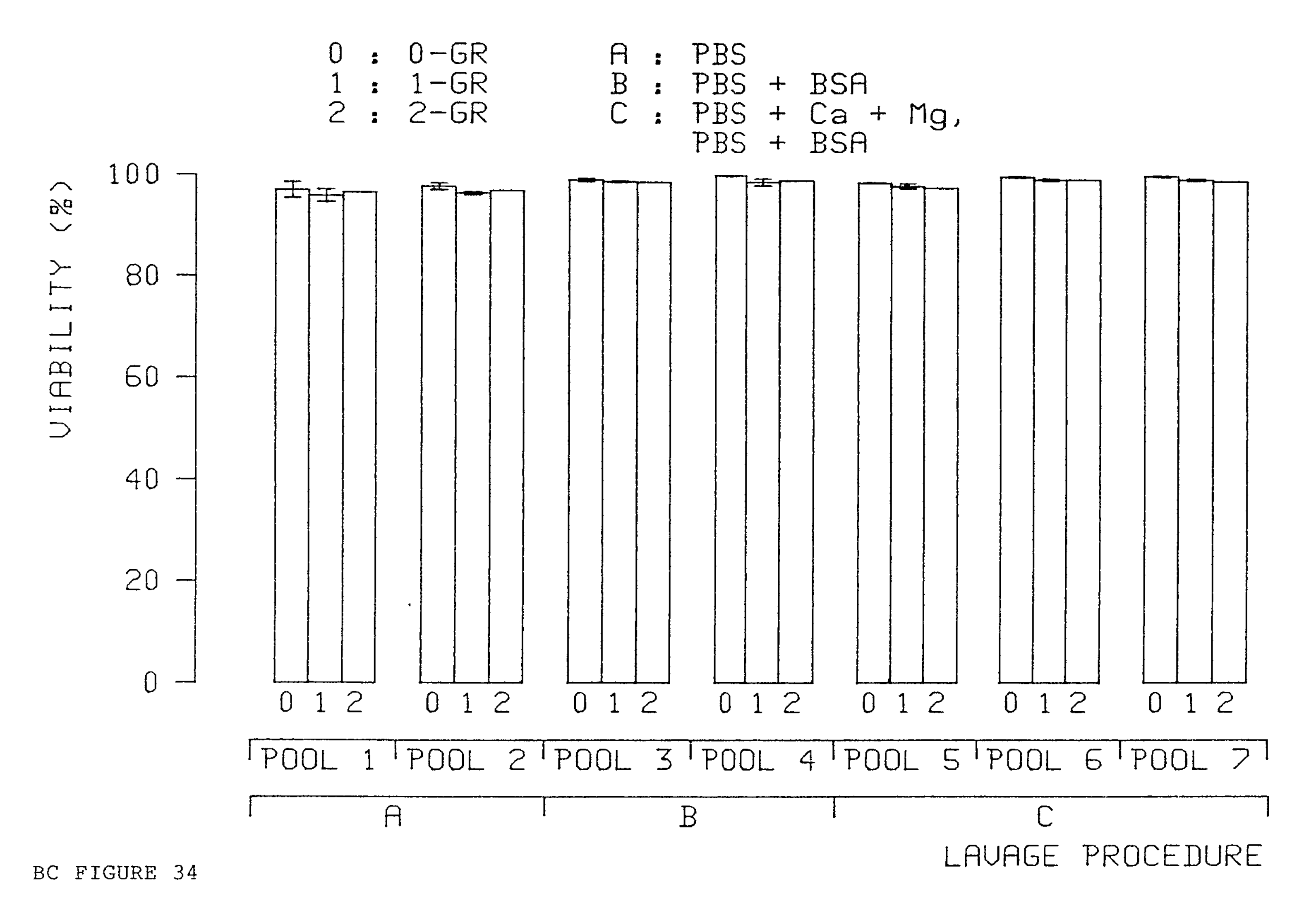
Remarks: for details see BC TABLE 38

A 0500/3057, H03228, TH, V129 F155 V72 R

2029028911

A 0500/3057, H03192, TH, V80 F155 V78, R

SUBREPORT P 0500/3057 GD151 (R) B23 WS M H03192



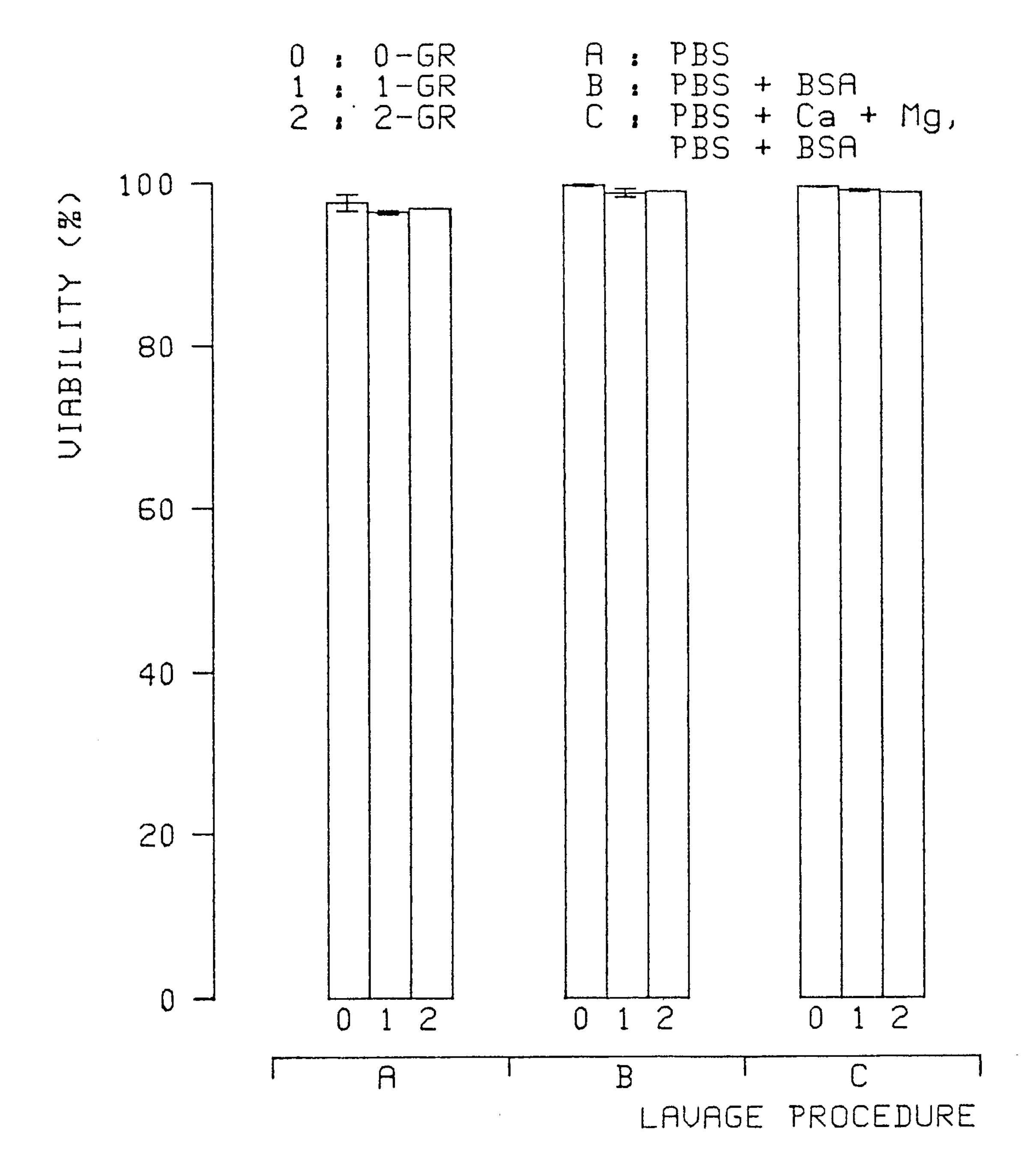

BC FIGURE 34

VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 40



2029028912




BC FIGURE 35

VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 40

A 0500/3057, HO3187, TH, U82 F155 U72, R

2029028913

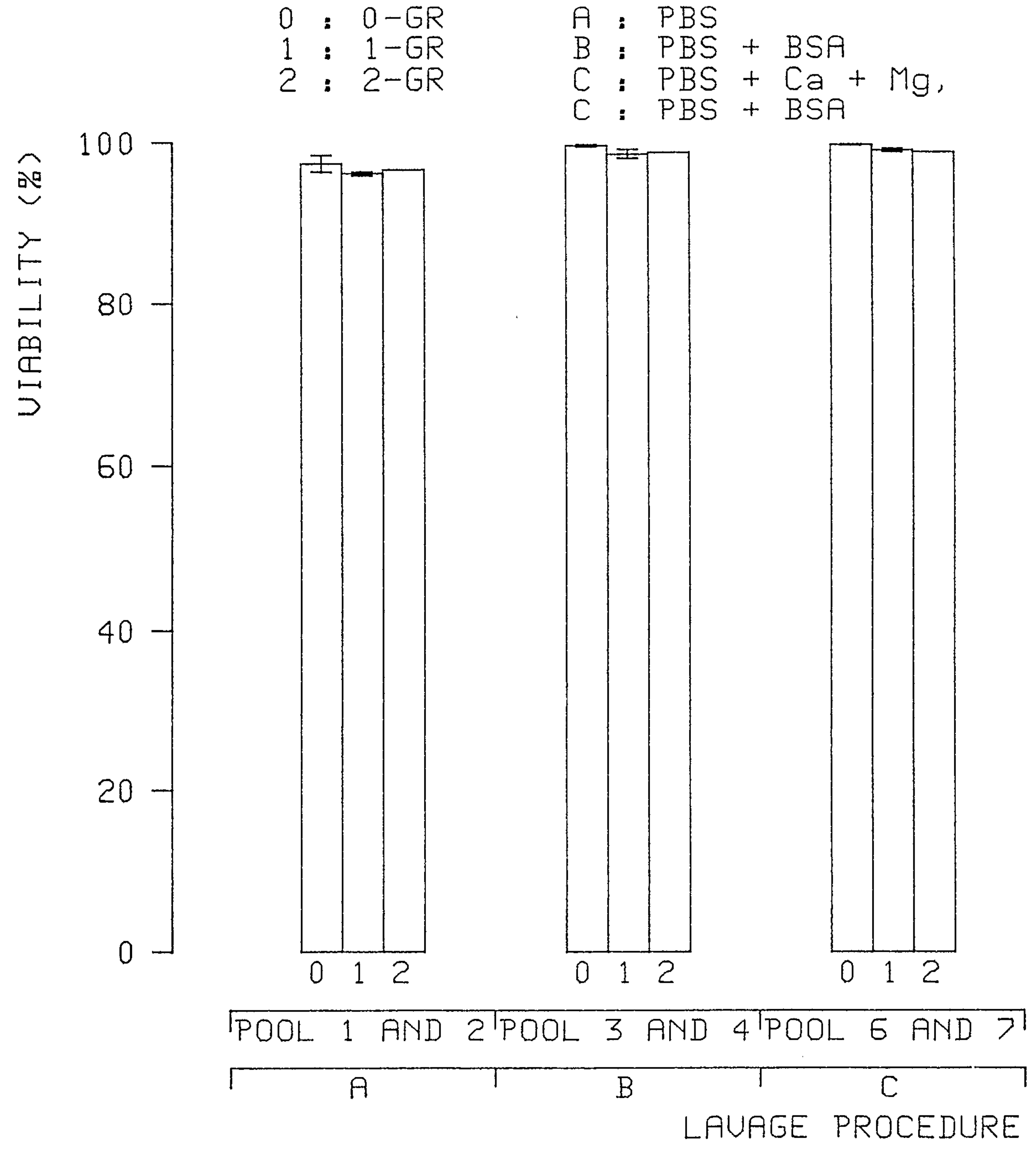
SUBREPORT P 0500/3057 GD151 (R) B24 WS M HO3472

BC FIGURE 35 (continued)

VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 40

2029028914




BC FIGURE 35 continued

VIABILITY OF MACROPHAGES, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 40

A 0500/3057, H03472, TH, V124 F155 V118 R

2029028915

SUBREPORT P 0500/3057   GD151 (R) B24   WS   M   HO3444

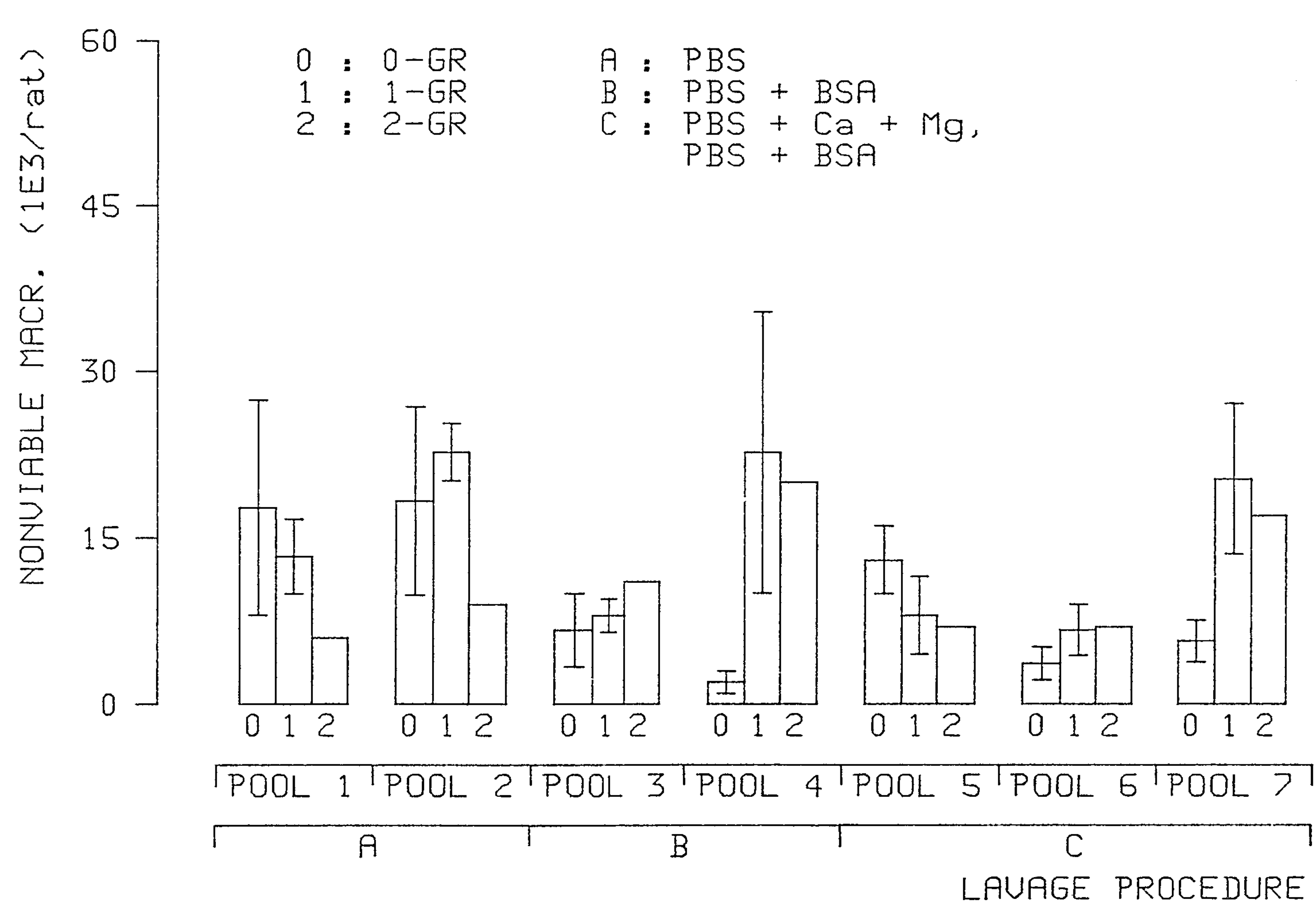
BC FIGURE 36

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 42

2029028916

A 0500/3057, H03444, TH, V133 F155 V89 R




BC FIGURE 36

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks : for details see BC TABLE 42

2029028917

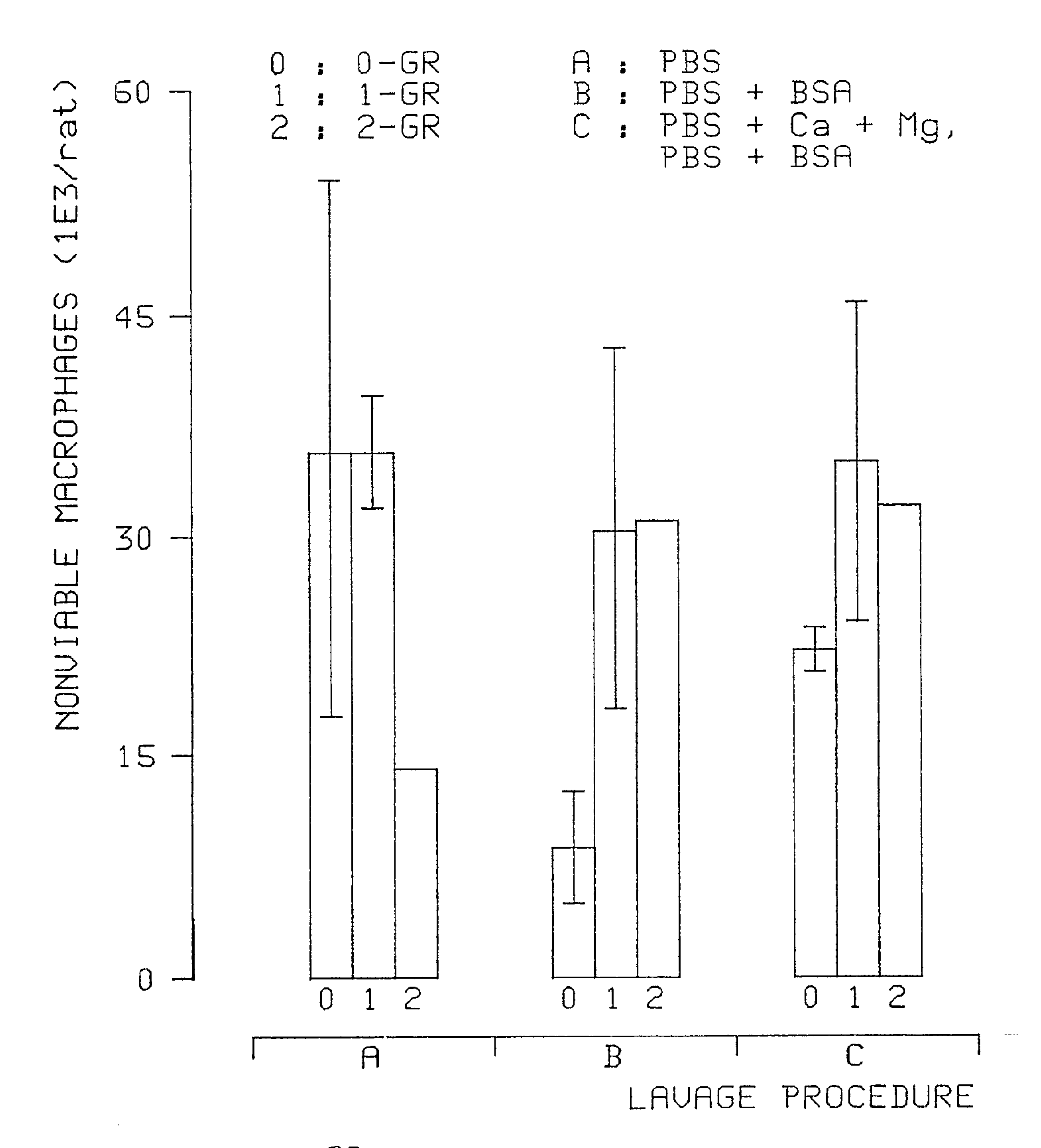
SUBREPORT P 0500/3057 GD151 (R) B24 WS M HO3445

BC FIGURE 37

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, SUM OF POOLS

Remarks: for details see BC TABLE 42

2029028918




BC FIGURE 37

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, SUM OF POOLS

Remarks : for details see BC TABLE 42

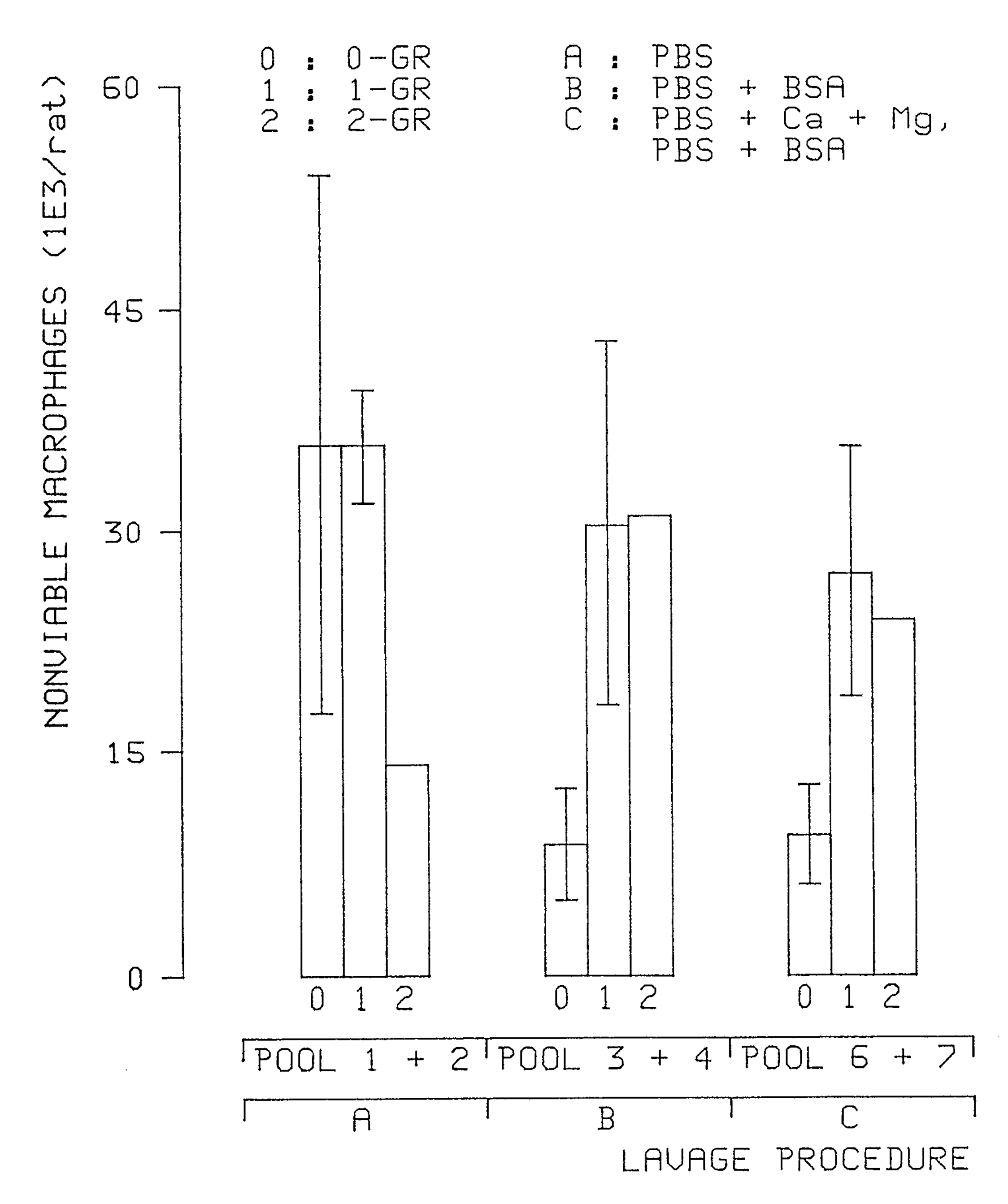
A 0500/3057, H03445, TH, V134 F155 V90 R

BC FIGURE 37 (continued)

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, SUM OF POOLS

Remarks: for details see BC TABLE 42

2029028920




BC FIGURE 37 continued

NUMBER OF NONVIABLE MACROPHAGES PER RAT, RESUSPENSION MEDIUM, SUM OF POOLS

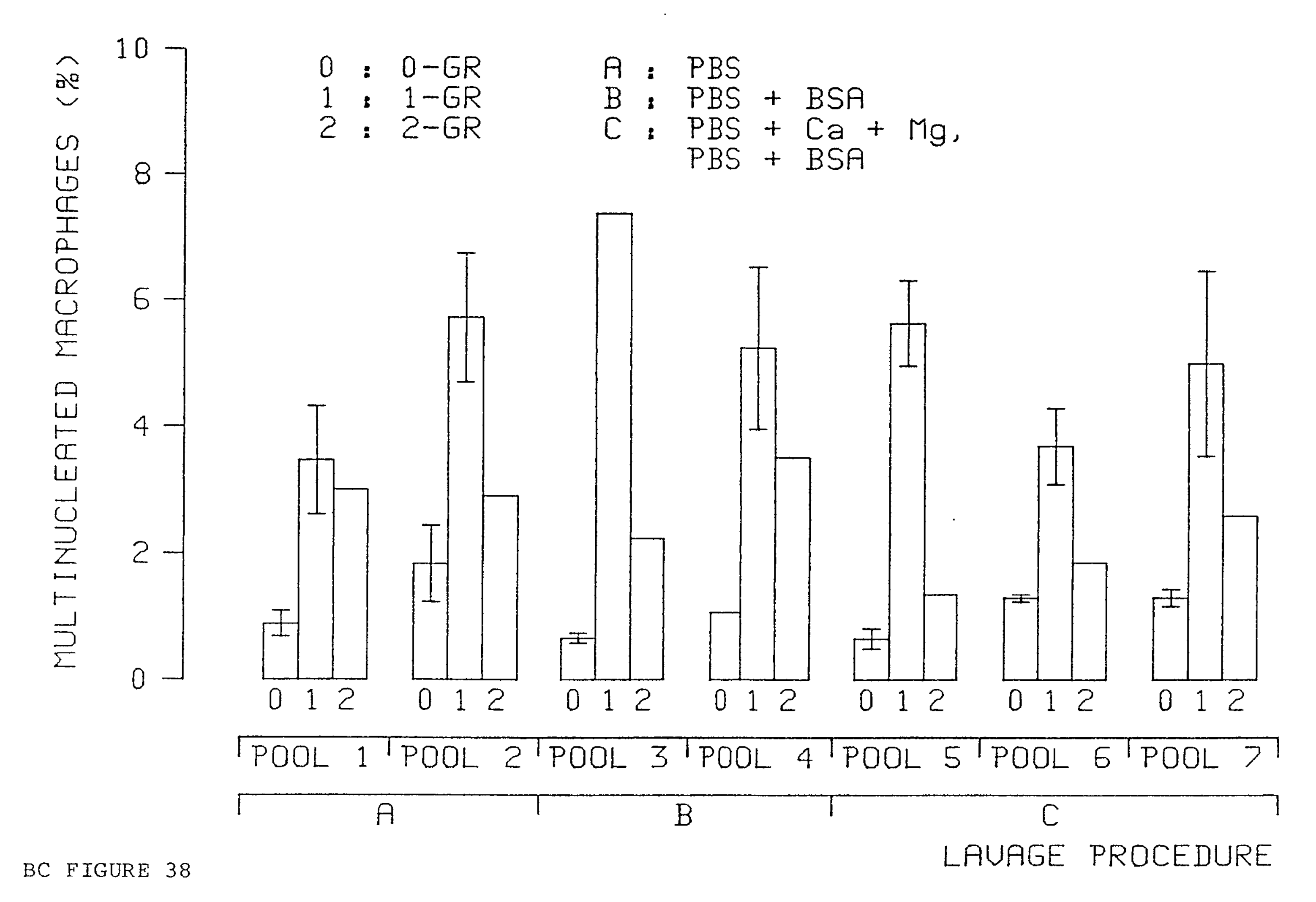
Remarks : for details see BC TABLE 42

A 0500/3057, H03446, TH, V135 F155 V91 R

2029028921

A 0500/3057, H03194, TH, V87 F155 V86, R

SUBREPORT P 0500/3057 GD151 (R) B25 WS M H03194

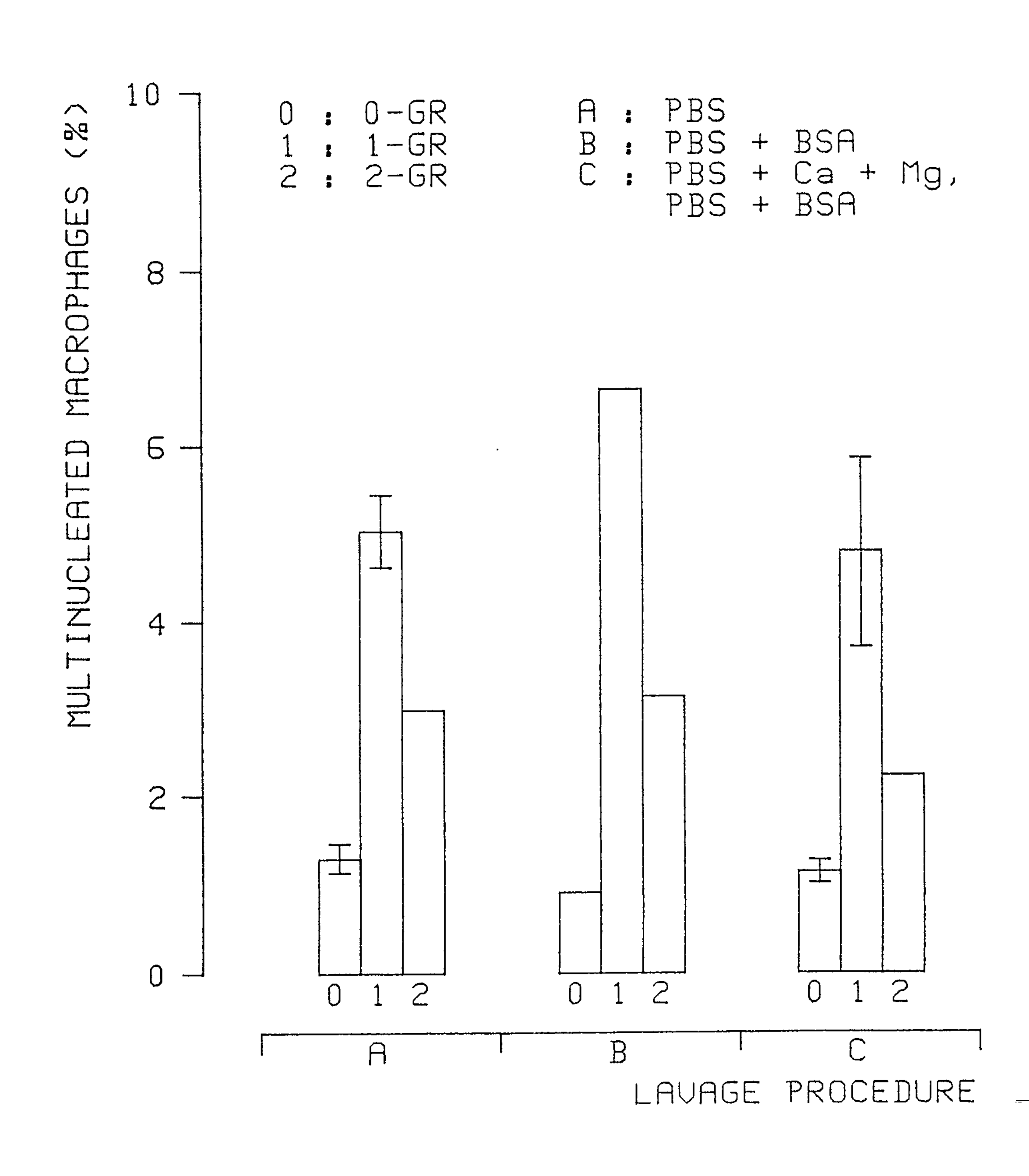



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BC FIGURE 38

RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 44




BC FIGURE 39

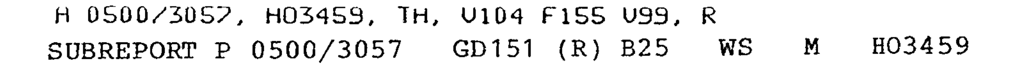
RELATIVE NUMBER OF MULTINUCLEATED MACROPHAGES, RESUSPENSION MEDIUM, CYTOCENTRIFUGE PREPARATION, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 44

A 0500/3057, H03198, TH, V88 F155 U79, R

2029028923

2284


H 0500/3057, H03459, TH, V104 F155 V99, R
SUBREPORT P 0500/3057 GD151 (R) B25 WS M H03459

MACROPHAGE AREA (um2)

500
400
300
200
100
0

0 : 0-GR
1 : 1-GR
2 : 2-GR

A : PBS
B : PBS + BSA
C : PBS + Ca + Mg,
PBS + BSA

0 1 2 0 1 2 0 1 2 0 1 2 0 1 2

POOL 1 | POOL 2 | POOL 3 | POOL 4 | POOL 5 | POOL 6 | POOL 7

A | B | C

LAVAGE PROCEDURE

BC FIGURE 40

MEAN MACROPHAGE AREA, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

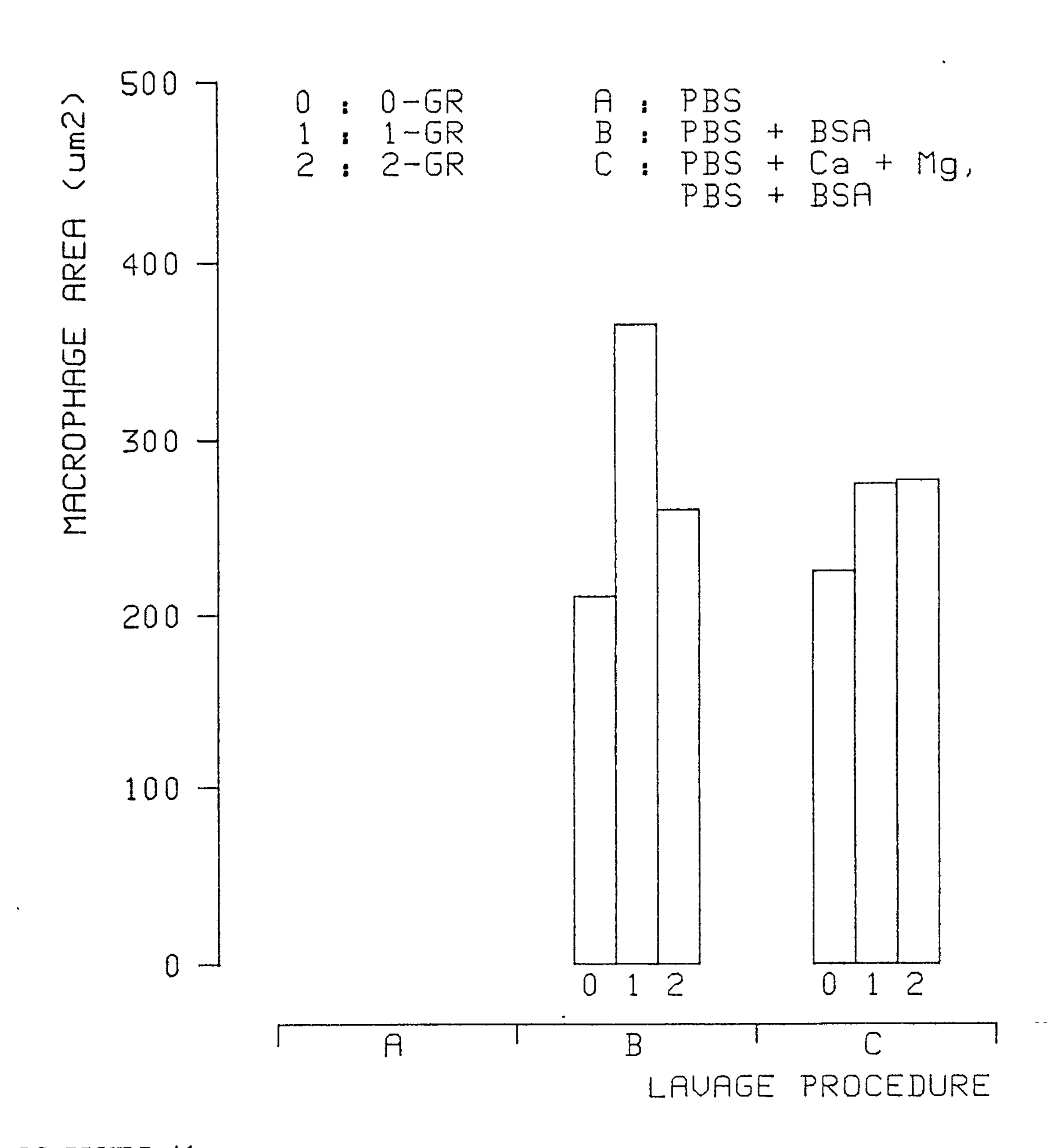
Remarks: for details see BC TABLE 47

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SUBREPORT P 0500/3057 GD151 (R) B25 WS M HO3460



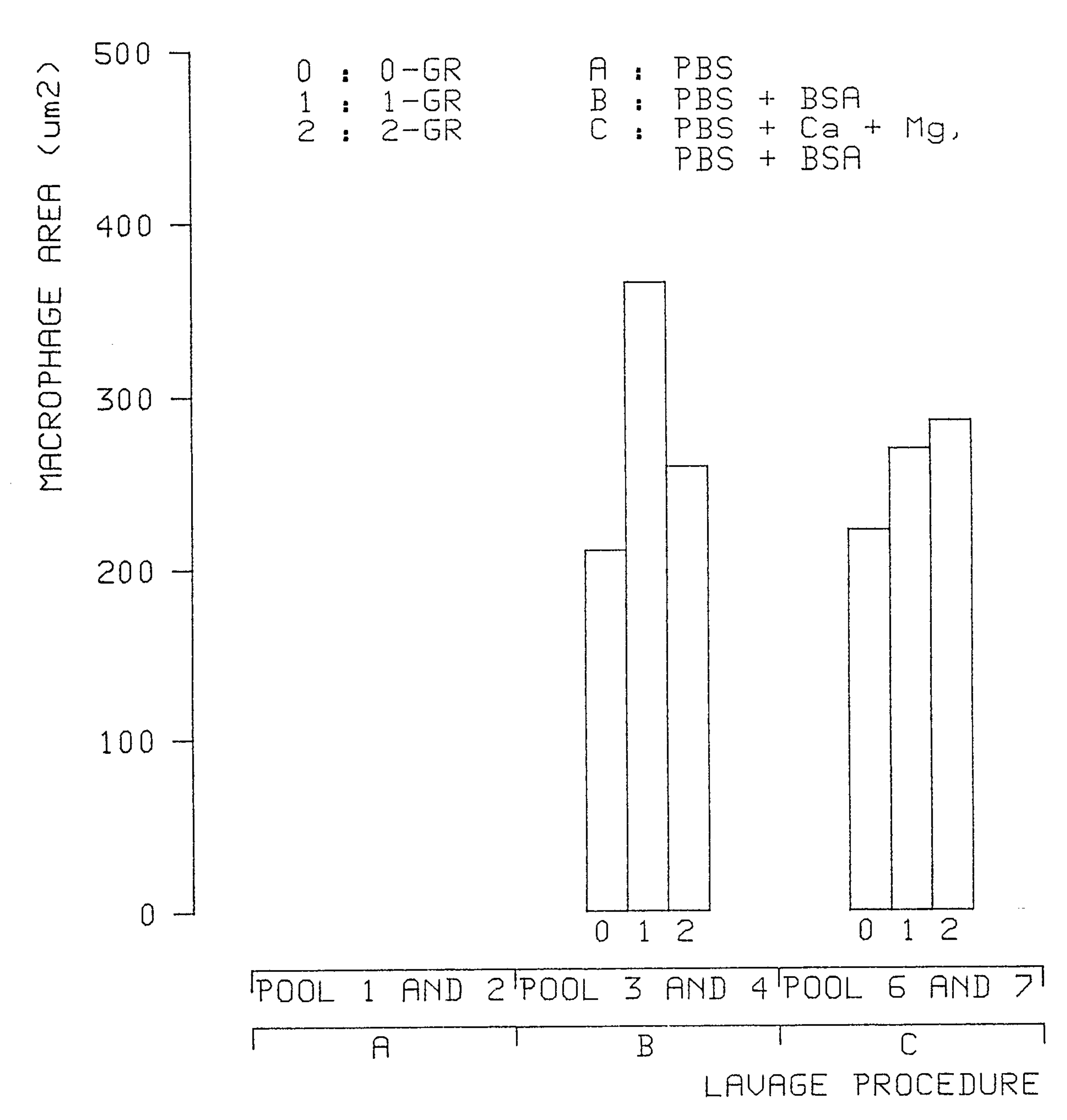

BC FIGURE 41

MACROPHAGE AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 47

A 0500/3057, H03460, TH, V105 F155 V90, R

2029028925



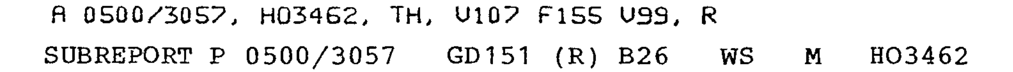

BC FIGURE 41 (continued)

MACROPHAGE AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 47

A 0500/3057, HO3461, TH, U125 F155 U106 R

2029028926


A 0500/3057, H03462, TH, V107 F155 V99, R
SUBREPORT P 0500/3057 GD151 (R) B26 WS M H03462

NUCLEUS AREA (um2)

90, 75, 60, 45, 30, 15, 0

0 : 0-GR
1 : 1-GR
2 : 2-GR

A : PBS
B : PBS + BSA
C : PBS + Ca + Mg, PBS + BSA

0 1 2 | 0 1 2 | 0 1 2 | 0 1 2 | 0 1 2

POOL 1 | POOL 2 | POOL 3 | POOL 4 | POOL 5 | POOL 6 | POOL 7

A | B | C

LAVAGE PROCEDURE

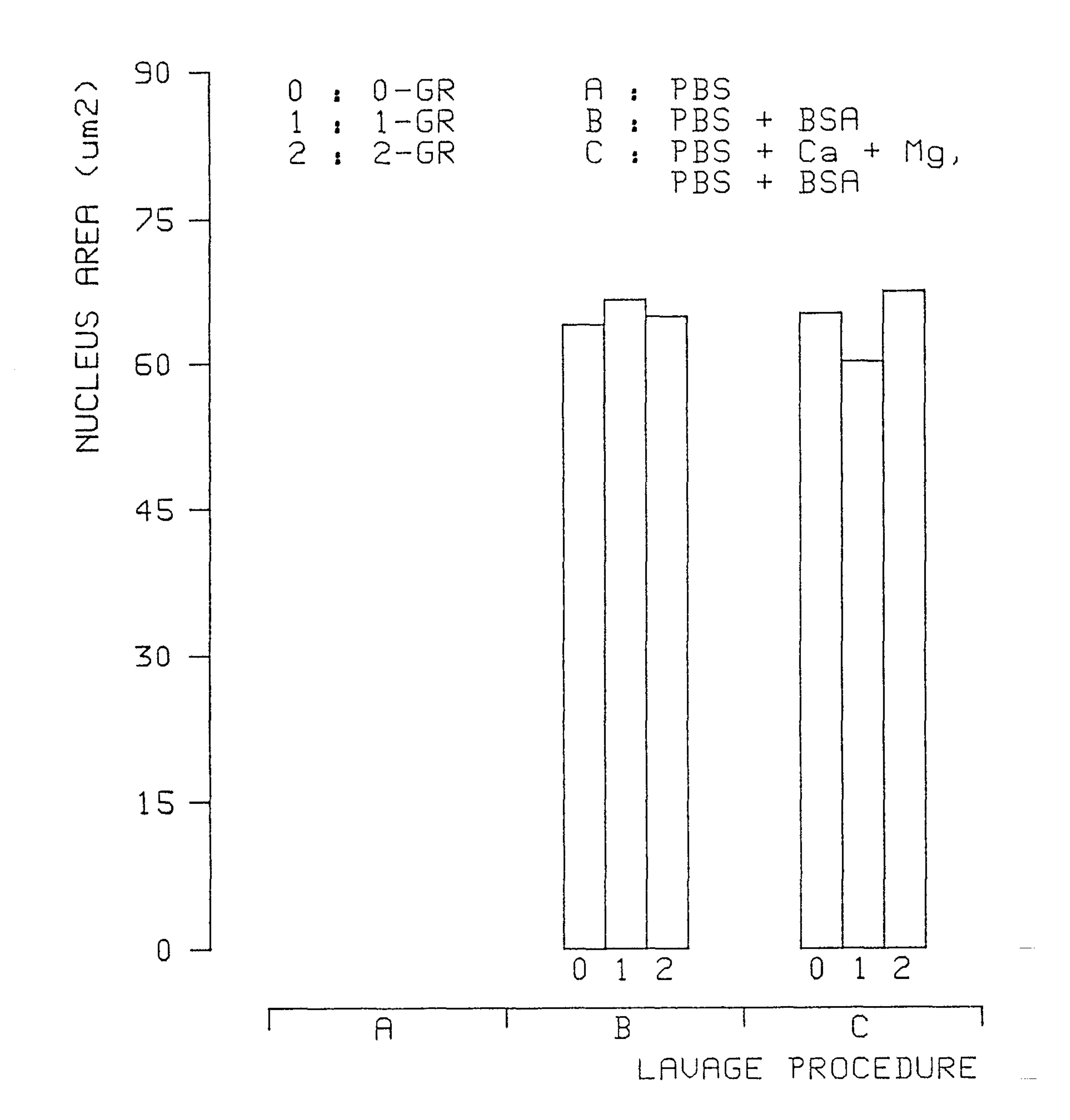
BC FIGURE 42

MEAN NUCLEUS AREA, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 49

2029028927

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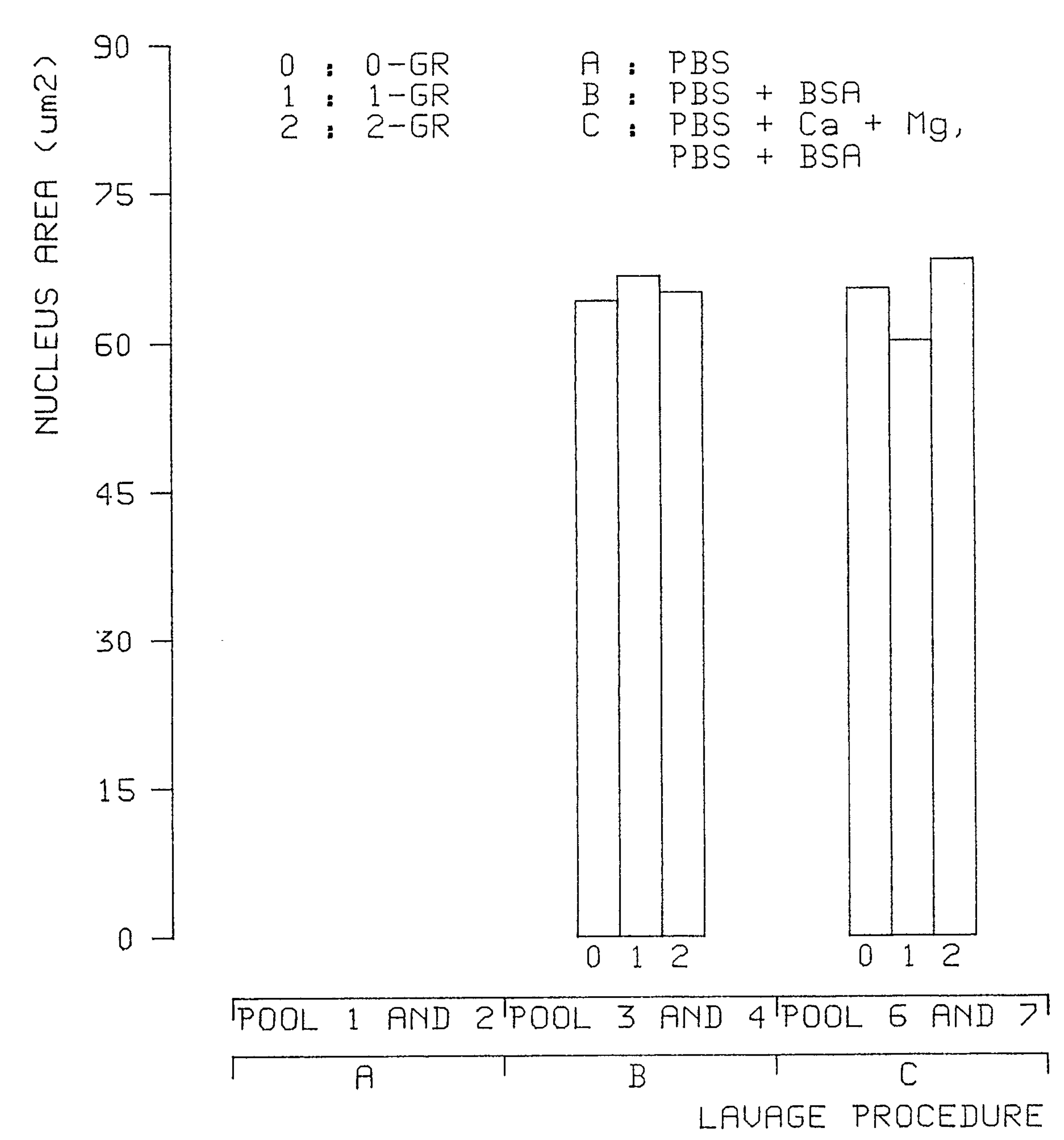

BC FIGURE 43

NUCLEUS AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 49

A 0500/3057, H03463, TH, V108 F155 V105, R

2029028928




BC FIGURE 43 (continued)

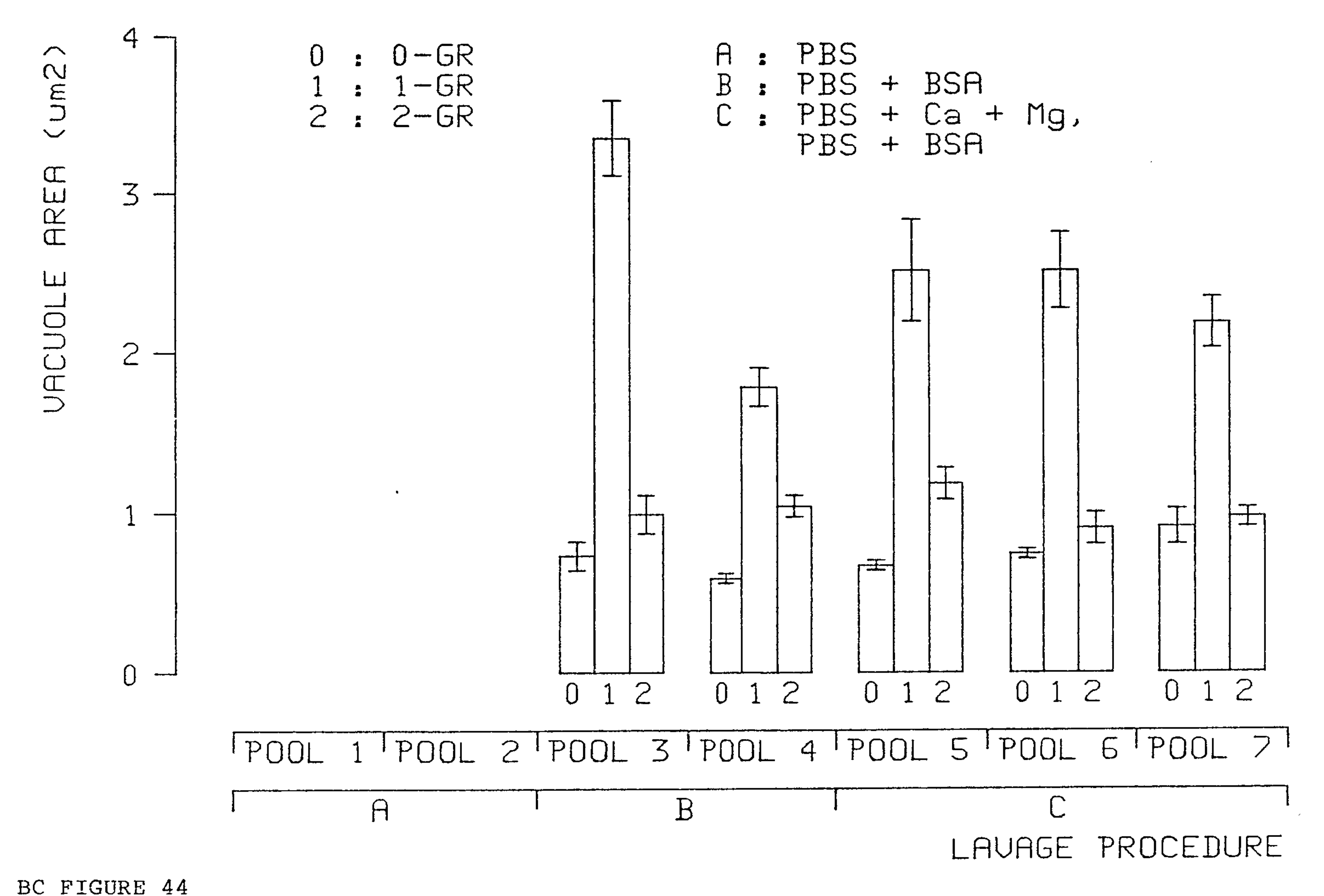
NUCLEUS AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 49

A 0500/3057, HO3464, TH, U126 F155 U109 R

2029028929

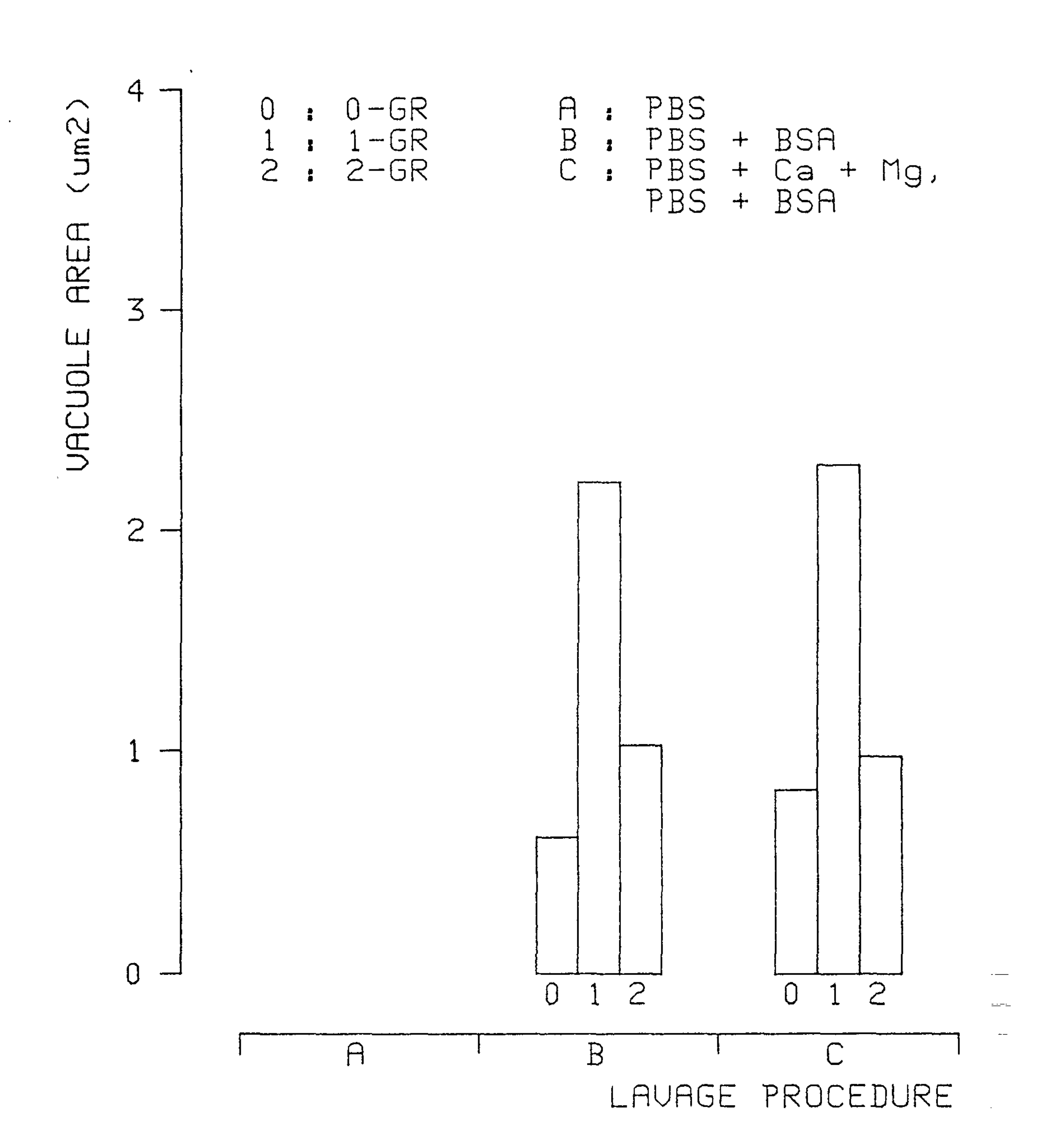
A 0500/3057, H03465, TH, V110 F155 V99, R
SUBREPORT P 0500/3057 GD151 (R) B26 WS M H03465




BC FIGURE 44

MEAN VACUOLE AREA, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 51



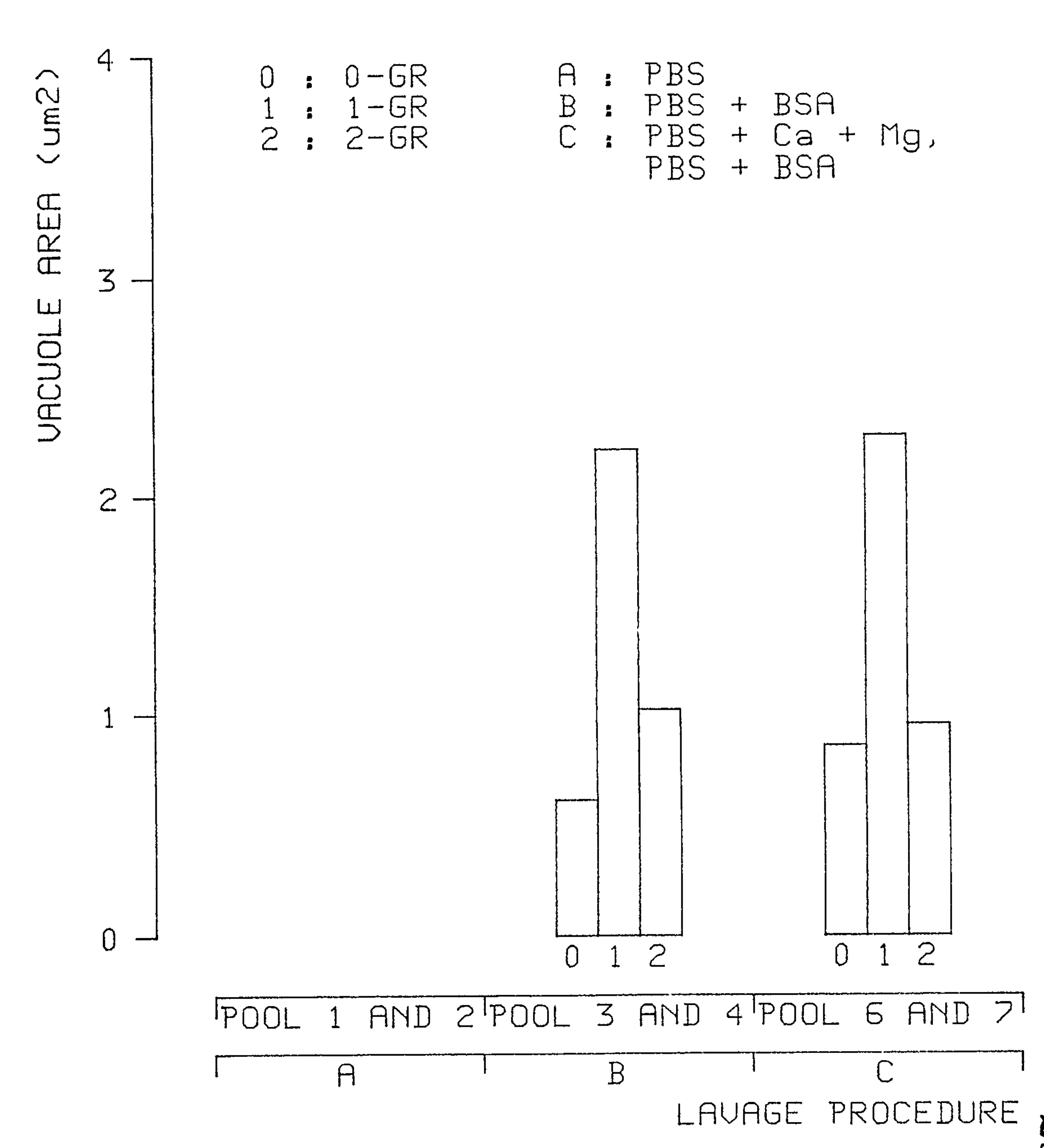

BC FIGURE 45

VACUOLE AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 51

A 0500/3057, H03466, TH, U111 F155 U108, R

2029028931




BC FIGURE 45 (continued)

VACUOLE AREA, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

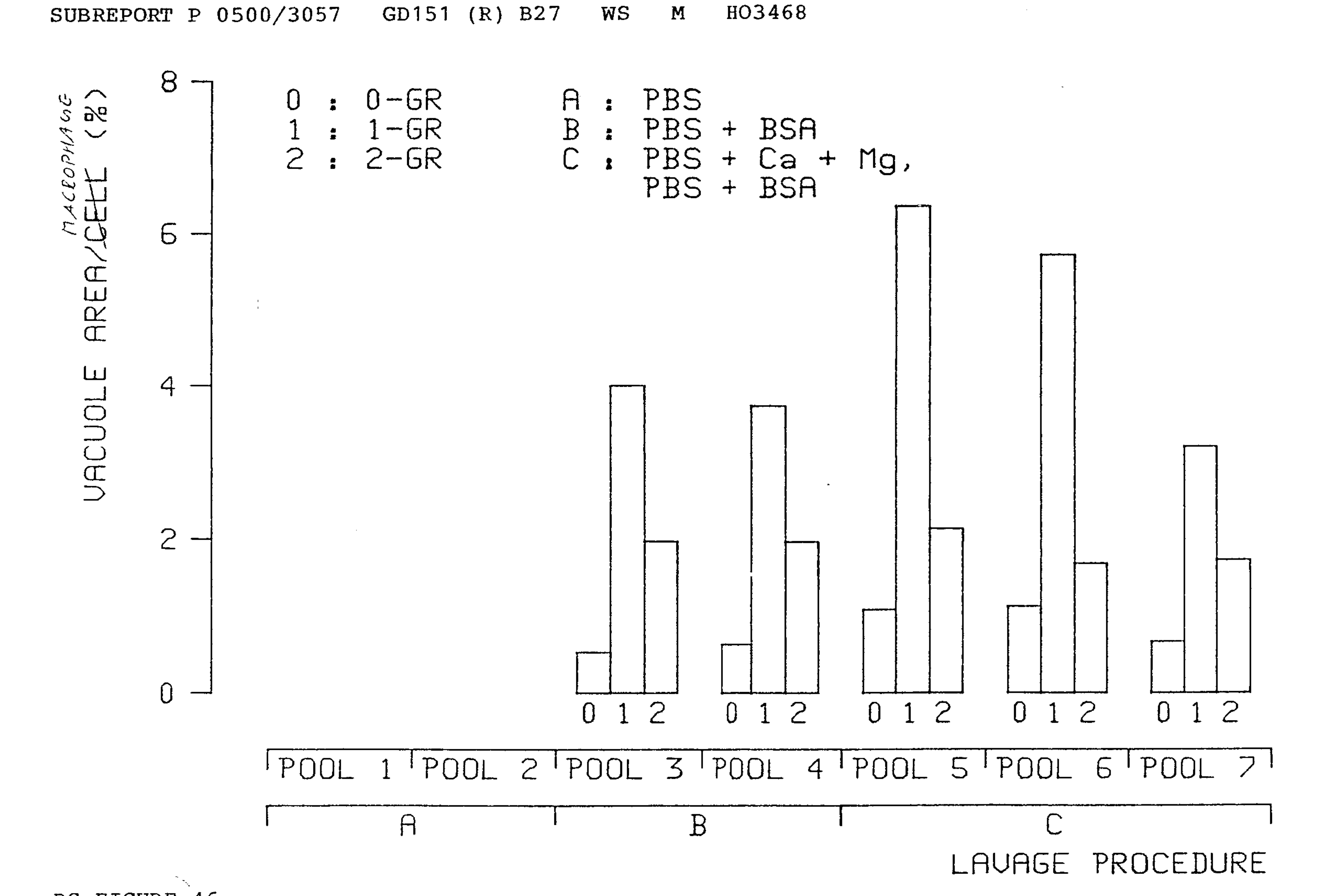
Remarks: for details see BC TABLE 51

A 0500/3057, HO3467, TH, U127 F155 U112 R

2029028932

A 0500/3057, H03468, TH, V113 F155 V110, R

SUBREPORT P 0500/3057 GD151 (R) B27 WS M H03468





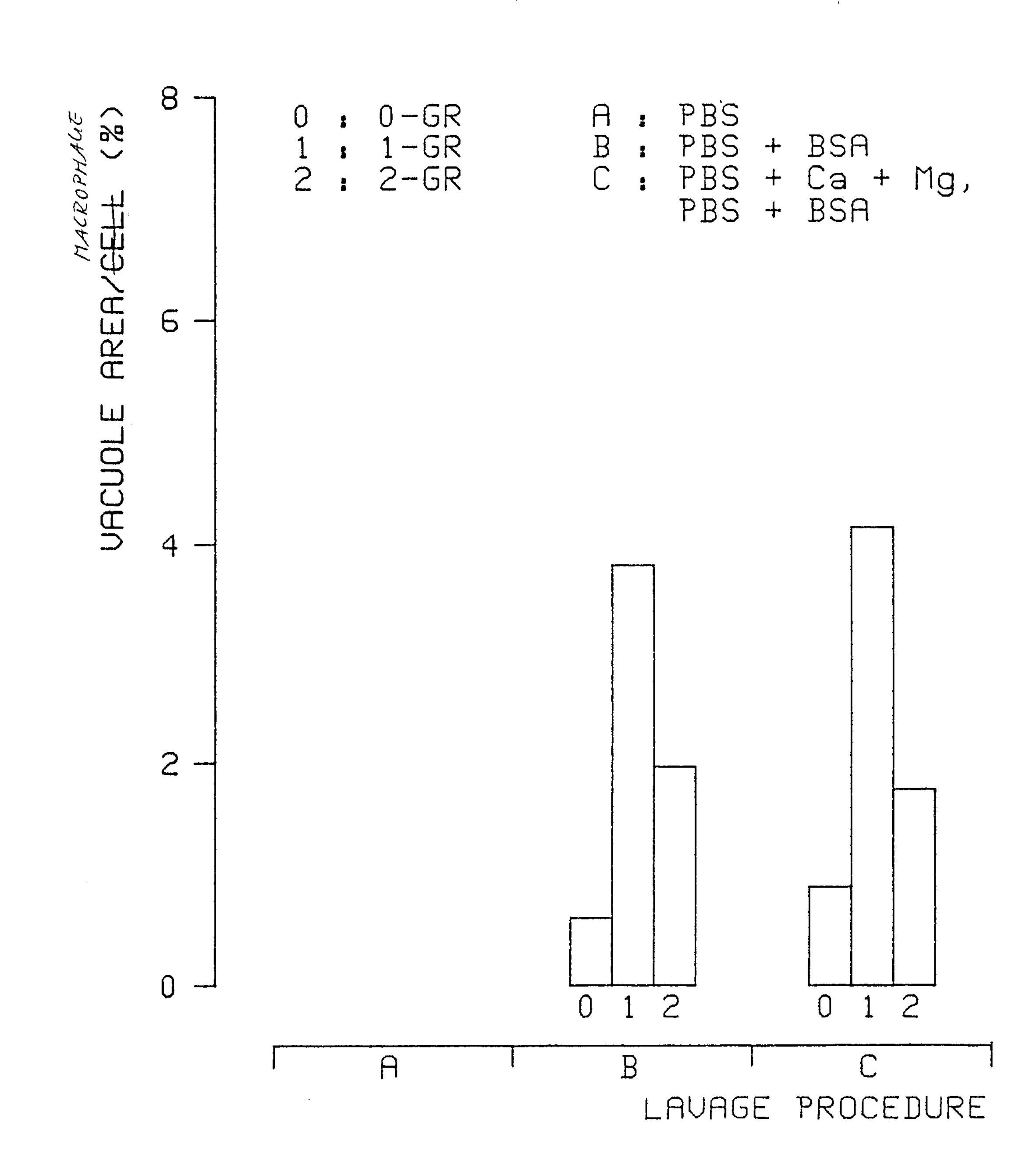

BC FIGURE 46

VACUOLE AREA PER ~~CELL~~ MACROPHAGE, RESUSPENSION MEDIUM, INDIVIDUAL POOLS

Remarks: for details see BC TABLE 52

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SUBREPORT P 0500/3057 GD151 (R) B27 WS M H03469




BC FIGURE 47

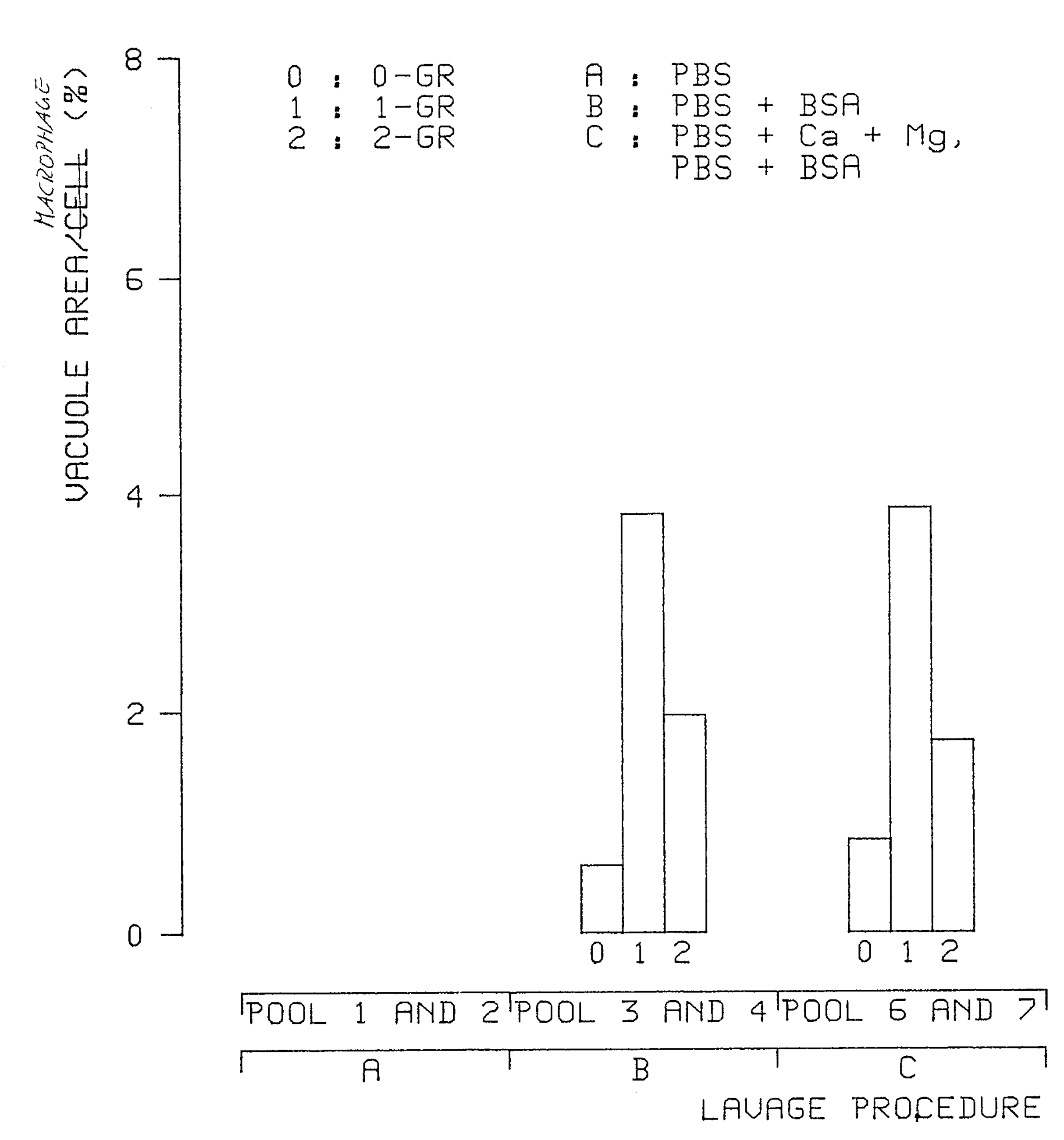
RELATIVE VACUOLE AREA PER ~~CELL~~ MACROPHAGE, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 52

A 0500/3057, H03469, TH, U114 F155 U111, R

2029028934

2284




BC FIGURE 47 (continued)
RELATIVE MACROPHAGE
VACUOLE AREA PER ~~CELL~~, RESUSPENSION MEDIUM, WEIGHTED MEANS OF POOLS

Remarks: for details see BC TABLE 52

A 0500/3057, HO3470, TH, V128 F155 V115 R

2029028935

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Epidermal ornithine decarboxylase activity after single dermal application of nicotine to NMRI mice,
Study Director : Dr.rer.nat. R. Mull
Study Codirector: -
issued: 11.Jul.83

INBIFO study A 0500/3013,
24-day smoke inhalation study on male rats with 2R1 and MW cigarettes and nicotine vapor added to non-tobacco smoke, biochemical and microbiological analyses,
Study Director : Dr.rer.nat. W. Reininghaus
Study Codirector: J. Kühl
issued: report in preparation

INBIFO study A 0500/3018,
? 91-day smoke inhalation study on male rats with 2R1 cigarette, response of bioassays to subacute inhalation recovery,
Study Director : Dr.rer.nat. W. Reininghaus
Study Codirector: J. Kühl
issued: 17.Mar.80

INBIFO study A 0500/3025,
21-day inhalation study with 2R1 standard reference cigarettes and BVW cigarettes on rats (PT),
Study Director : Dr.rer.nat. W. Reininghaus
Study Codirector: J. Kühl
issued: proposal in preparation

INBIFO study A 0500/3047,
21-day smoke inhalation study with mainstream and sidestream cigarette smoke of standard reference cigarette 2R1 on rats,
Study Director : D. Becker
Study Codirector: Dr.rer.nat. R.-A. Walk
issued: report in preparation

INBIFO study A 0500/3056,
21-day smoke inhalation study with cigarette smoke of standard reference cigarette type 2R1 on Sprague Dawley rats, sister chromatid exchange and chromosome aberrations in peripheral blood lymphocytes,
Study Director : D. Becker
Study Codirector: Dr.rer.nat. R.-A. Walk
issued: 3.Jan.83

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